STUDIES ON THE STEREOSPECIFICITY OF CLOSELY RELATED COMPOUNDS

by

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CONTENTS

ABSTRACT

Abstract

Esters of dimethylamino-, diethylamino-, pyrrolidino- and piperidino- ethanol have been prepared with the resolved forms of mandelic, cyclohexylphenylacetic, cyclohexylphenylglycollic and α-methyltropic acids and converted to their hydrochlorides, methiodides and ethiodides. The resolved forms of hyoscyamine and hyoscine have been quaternised with methyl, ethyl, n-propyl and n-butyl iodides and the resolved forms of homatropine have been quaternised with methyl and ethyl iodides. In all 54 enantiomeric pairs have been synthesised.

The affinity constants of all the compounds have been measured for postganglionic parasympathetic acetylcholine receptors of the isolated guinea-pig ileum. Each was tested in at least two different conconcentrations and appeared to be acting competitively. Experiments were also made with mixtures of the enantiomers of cyclohexylphenylglycolloylcholine iodide and the results indicated that the isomers competed with each other and with the agonist for the receptors. Four pairs of enantiomers were tested on the isolated guinea-pig bronchial strip preparation and one pair on isolated guinea-pig iris and their affinity constants calculated for the acetylcholine receptors in these tissues. The results have been used in attempts to see to what extent these receptors are similar in structure.

The values of the affinity constants (K) of enantiomers can be used to calculate the stereospecific index (S.S.I.), that is the ratio of the activities of the enantiomers, but it is more convenient to use the difference in values of log K, which is log S.S.I., because this is directly proportional to the difference in the free energy of adsorption. In the series of enantiomers derived from any one acid log S.S.I. is not constant, and the differences are greater than the experimental

error, so altering the composition of the onium group disturbs the binding of the acid part of the ester.

With esters of cyclohexylphenylglycollic and α-methyltropic acid and with the hyoscyamine and hyoscine derivatives there is considerable stereospecificity (log S.S.I. up to 2.5, S.S.I. up to 300) but with the esters of mandelic and cyclohexylphenylacetic acid there appears to be only slight stereospecificity (log S.S.I. up to 0.6, S.S.I. up to 4). The overall range in values of log S.S.I. in all the series, however, was around 0.8 log units.

More information is obtained, however, from comparisons of values of log K, rather than from the values of log S.S.I. derived from them. From these, and from earlier values of log K, for compounds lacking an asymmetric centre, the biggest effect a group has on binding has been calculated, together with the disturbances in binding which take place when this group is introduced into other compounds. These values can be used to predict, very roughly, the affinities of new compounds.

Provided measurements of affinity really are made at equilibrium, as they should have been here, there is no special value in using series of enantiomeric pairs rather than any other series, unless the disturbances in binding arise primarily from the effects a group has on the preferred conformation of the antagonist, which should be the same in both enantiomers, rather than from conformations in the complex with the receptor, which will be different.

INTRODUCTION

Introduction

The discovery that biological systems could differentiate between enantiomeric pairs of compounds was made by Pasteur (1858). He showed that (+) ammonium tartrate was destroyed more rapidly than the (-) isomer by a mould, Penicillium glaucum.

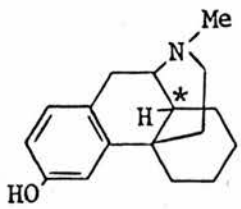
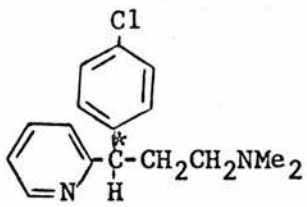
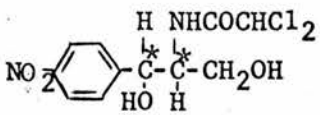
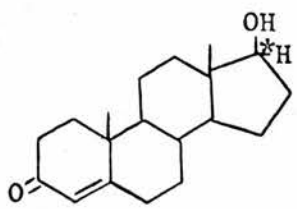
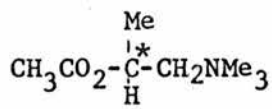
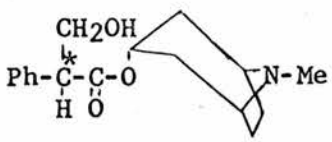
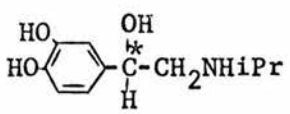
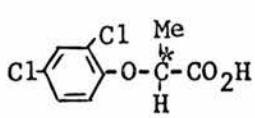
Since this initial discovery, many more examples of the stereospecific interaction of certain small molecules with living systems have been cited. Some of these are shown in table 1 which illustrates the wide range of compounds which act in this way. It is convenient to express the results as the ratio of the activities of the enantiomers and this is often referred to as the stereospecific index.

If it is intended to draw conclusions about the stereochemical requirements for the binding of drugs to receptors, two things must be remembered:

1) Results obtained from whole animal experiments will be influenced by factors such as absorption, distribution, metabolism, uptake and excretion. Any one or more of these processes may or may not be stereospecific or rate limiting. Suppose absorption was stereospecific and rate limiting: the drug would then appear to be stereospecific. If however this step was not rate limiting, then the drug might not appear to be acting in a stereospecific way. The final stereospecific index obtained from an experiment such as this will depend on all these variables and may not indicate whether the binding of the drug to its receptor is stereospecific or not. The use of isolated preparations largely removes such difficulties, but a further problem remains:

2) Many of the compounds which are stereospecific are agonists i.e. drugs which elicit some sort of response from the system on which they

Table 1

Type of compound: Example	Structure	Stereo-specific index	More active form
Morphine-like Analgesics Levorphanol, Dextorphan		90	(-)
Antihistamines Chlorpheniramine		240	(+)
Antibiotics Chloramphenicol			R(-) threo
Androgenic Hormones Testosterone		27	17β OH
Acetylcholine-like Agonists Acetyl-β-methyl choline		240	S(+)
Acetylcholine-like Antagonists Atropine		220	S(-)
Adrenergic agonists Isoprenaline		1600	(-)
Plant Growth Regulators α (2,4 dichlorophenoxy) propionic acid			(+)

Reference asymmetric carbon *

Values based on results from a review by Casy (1970) and references cited there.

act. The stimulus which causes the response is a function of the affinity and of the efficacy of the agonist (Stephenson, 1956). An alteration in the stereochemistry of an agonist may affect its affinity or its efficacy or both and it is very difficult to decide which of these has occurred.

To obtain information about drug-receptor binding from stereospecificity studies, isolated preparations and compounds which act as antagonists must be used. The measurement of affinity must also be carried out under conditions which ensure that the antagonist is in equilibrium with its receptors. If this is not done any differences in the rates of diffusion of the enantiomers will affect the values of the stereospecific index obtained.

There are really only two types of drug which can conveniently be studied in this way; the antihistamines, and substances, like atropine, which block parasympathetic postganglionic acetylcholine receptors. Many antihistamines however behave as non-competetive antagonists and this complicates matters.

It has been known for a long time that certain antagonists bind stereospecifically to postganglionic parasympathetic acetylcholine receptors. Cushny (1921) found that the resolved forms of hyoscine differed 15-fold in their ability to abolish pilocarpine induced contractions of rabbit ileum, the (-) isomer being more active. He deduced from these and other results that such differences were due to differences in the binding of the enantiomers to the receptors (Cushny 1926). Because these results with hyoscine were obtained on an isolated preparation, this deduction was valid. The true value of the stereospecific index however may well be greater than 15 for the following two reasons:

1) The antagonism was estimated only two minutes after the blocking drug had been added to the preparation. It is unlikely that equilibrium could have been established between the antagonist and the receptors in so short a time, especially in the case of the more active isomer.
2) As pointed out by Ing (1955), the resolution of alkaloids such as hyoscine is often difficult but their racemisation in solution occurs very easily. It is possible then, that the material tested by Cushny was not completely resolved to begin with, or had racemised to some extent in the solution which was used.

Nevertheless, Cushny's results indicated a definite stereospecificity in the binding of antagonists to the postganglionic parasympathetic acetylcholine receptors. His interpretation of these results in terms of the binding of certain groups in the drug to complementary structures in the tissue, however, were not put formally until Ogston (1948) came to similar conclusions about the binding of substrates to enzymes.

The enantiomers of the related alkaloid, hyoscyamine, are further examples of antagonists which bind stereospecifically to the postganglionic parasympathetic acetylcholine receptors. Marshall (1955) found that the stereospecific index was 30 on guinea-pig ileum, and Long and others (1956) obtained the value 220 on rabbit ileum.

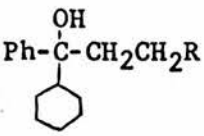
Hyoscine and hyoscyamine are esters of tropic acid with scopine and tropine respectively. The amino-alcohols both have a plane of symmetry and are optically inactive. The absolute configurations of the enantiomers of tropic acid are known and it is the (-) S isomers of hyoscine and hyoscyamine which have the greater affinity for the receptors.

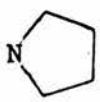
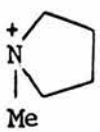
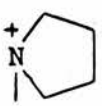
In contrast it appeared that the enantiomers of the related compound homatropine (mandelyl tropine) do not differ greatly in

activity. Cushny (1920) found that the (-) isomer was only about twice as active as the (+) form in antagonising salivation in dogs. The low stereospecificity could, of course, be due to the *in vivo* nature of the test, though this detected differences between the isomers of hyoscine and hyoscyamine. It could also have been due to incomplete resolution.

Interest in synthetic atropine-like compounds increased greatly during World War II because of the need for substances to counteract the lethal effects of nerve-gases. Most of these drugs were based on modifications of the structure of acetylcholine, rather than on that of the established antagonists hyoscine and hyoscyamine, and some of them contained an asymmetric carbon atom and have been resolved. Among later developments were propylamine derivatives including such compounds as benzhexol and procyclidine which were resolved by Adamson and Duffin (1957). These and their metho and etho salts were tested by Duffin and Green (1955) and the results which included in vitro estimates (table 2) suggested that the stereospecific index was dependent upon the nature of the onium group, even though this was situated at the other end of the molecule from the asymmetric centre. Changes at one part of the molecule therefore appeared to disturb the binding to the receptors of other parts, even when far distant, which is interesting because this idea has been suggested from other work, not involving optically active compounds, by Akramson and others (1969). The conclusion should be treated with caution however because Long and others (1956) found that the stereospecific index for benzhexol on rabbit ileum was 160. So it is possible that the sample of benzhexol used by Duffin and Green was not adequately resolved.

Table 2

Activity and stereospecific index on guinea-pig ileum of the resolved forms of 

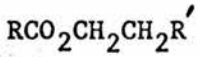
(R)-		Equipotent molar ratio relative to atropine	Stereospecific index
	(+)	540	49
(±) is Procyclidine	(-)	11	
	(+)	81	160
(±) is Tricyclamol	(-)	0.51	
	(+)	230	290
	(-)	0.79	
	(+)	13.7	9.8
(±) is Benzhexol	(-)	1.4	
	(+)	45	48
	(-)	0.94	

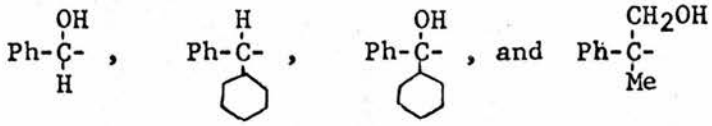
In fact the effect of altering one part of the molecule on the stereospecificity arising from a distant asymmetric centre cannot be assessed by making series of related compounds and resolving them because it is impossible to be sure that resolution is complete (or equally incomplete) for all the enantiomeric pairs. To obtain any useful information it is necessary to start with resolved material and prepare series of compounds from this by reactions which do not involve racemisation. This has so far only been attempting in a small way. Scarselli, Cignarella and Maffii (1964) tested a few esters and amides of the resolved forms of α-methyltropic acid.

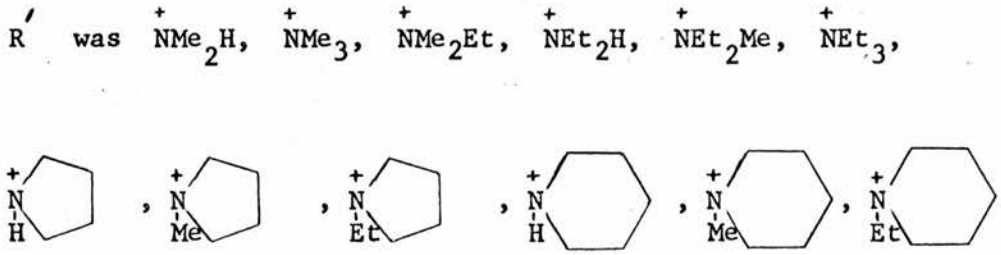
Ellenbroek and others (1965) made choline and β-methylcholine esters of the resolved forms of cyclohexylphenylglycollic, α-methyltropic, O-acetylmandelic, and phenyl-2-thienylglycollic acid and measured their pA_2 values. Recently Brimblecombe and others (1971) have made further esters of the resolved forms of cyclohexylphenylglycollic acid and determined their affinity constants, but only the hydrochlorides and methiodides of the N,N-dimethylamino-ethyl and N-methyl-piperidin-4-yl esters were made.

The aim of the present work has been to extend this rather limited study, in which only small changes in the onium group have been made, to include series of compounds all obtained from the same resolved material and having a large range of substituents in the onium group. This has mainly been done by esterifying the resolved forms of various optically active acids with different amino-alcohols and then making the tertiary, and metho- and etho-quaternary, salts of the products.

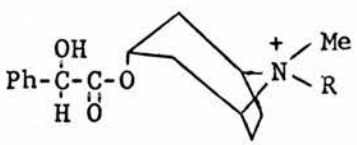
Most of the compounds can be represented by the general formula



Where R was 

and R′ was 

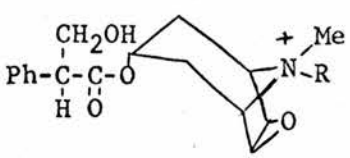
The resolved forms of homatropine and their metho and etho salts were also made.



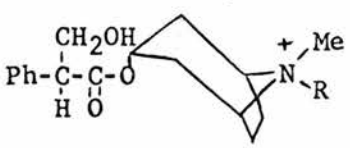
R = H, Me, Et

The resolved forms of hyoscine and hyoscyamine have also been quaternised with various alkyl groups.

Hyoscine



Hyoscyamine



Where R = H, Me, Et, nPr, nBu.

All the compounds behaved as competitive antagonists of acetylcholine at the postganglionic parasympathetic receptors in guinea-pig ileum, so it was possible to measure their affinity constants for these receptors. The method used was that of Edinburgh Staff (1968). In this test suitable contractions of a piece of guinea-pig ileum are obtained with two concentrations of carbachol, the bathing fluid is then replaced by bath fluid containing a known concentration of antagonist and the concentrations of carbachol are increased so as to produce contractions which are approximately the same size as those produced initially. The ratio of the concentrations of agonist producing contractions of equal size in the presence and absence of antagonist is called the dose-ratio. If the antagonist is competitive, this is related to the affinity constant of the antagonist by the equation

$$DR-1 = B.K_B \qquad \text{(Gaddum, 1937)}$$

Where DR is the dose-ratio

B is the concentration of antagonist

K_B is the affinity constant of the antagonist

If the antagonist competes with the agonist for the receptors and the dose-ratio is measured in conditions of equilibrium, the affinity constant should vary only with temperature and should be an absolute measure of the binding of the antagonist to the receptors. In these experiments it is the antagonist which requires time to come into equilibrium with the receptors but it is easy to see when this occurs because the effect of the antagonist becomes steady. It is in fact the logarithm of the affinity constant which is related

to the free energy of adsorption by the vant Hoff relationship

$$-\Delta G = RT \ln K$$

and the results are often expressed as values of log K rather than of K.

The variance of the estimates is assumed to be log normally distributed (Gaddum 1945). For a pair of optical isomers the log of the stereospecific index is given by the difference between the two values of log K so the errors in estimating the log of the stereospecific index will be about twice as great as those involved in measuring a single value of log K.

All the compounds were tested on the guinea-pig ileum but some have also been tested on the guinea-pig bronchial strip and on the isolated intact guinea-pig iris preparation. In the latter tests the agonist was carbachol so the antagonists were considered to be blocking postganglionic parasympathetic receptors, but the response of the iris to the agonist was slower than that of the ileum and the response of the bronchial muscle very much slower. A comparison of the affinities of various antagonists for the receptors in these tissues might therefore indicate whether the receptors were different from each other and from those in the ileum.

EXPERIMENTAL

Experimental

Synthesis of compounds

The compounds which have been made are of two types. In the first an optically active acid has been resolved and esterified with various substituted amino alcohols. In the second hyoscine and hyoscyamine have been resolved and quaternised with various alkyl halides.

The final products, quaternary compounds or the salts of tertiary bases, were recrystallised to constant melting point, and most were checked for chromatographic homogeneity on paper in n-butanol-ethanol-water (5:5:2) developed with a modified Dragendorff reagent (Thies and Reuther, 1954).

Analyses for ionic halogen were determined gravimetrically on samples of 50-150 mg.

Rotations, usually at 589 nm (sodium D line) and 300 nm were measured with a Bellingham and Stanley model B spectropolarimeter.

Melting points were determined with a Mettler FP1 instrument connected to a pen-recorder.

The compounds made are shown in tables 3 and 4

This account is divided into

a) The resolution of the acid or base

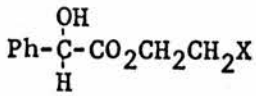
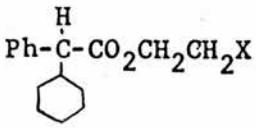
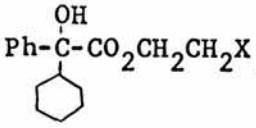
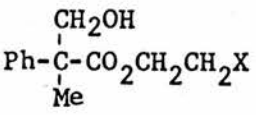
b) The formation of derivatives.

Resolution of the acids

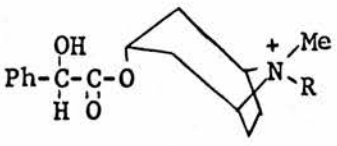
i) The resolved forms of mandelic acid were bought from A.G. Fluka and had:-

	m.p.	
R	130-3°	$[\alpha]_D^{21}$ -158° (c = 2, water)
S	130-3°	$[\alpha]_D^{21}$ +159° (c = 2, water)

Table 3

General Formula	Form	(X) = Onium Group with R = H, Me, Et			
					
	(±)	Me,Et	Me,Et	Me,Et	Me,Et
	R		" "	" "	" "
	S		" "	" "	" "
	(+)	H,Me,Et	H,Me,Et	H,Me,Et	H,Me,Et
	(-)	" " "	" " "	" " "	" " "
	R	" " "	" " "	" " "	" " "
	S	" " "	" " "	" " "	" " "
	(±)	" " "	" " "	" " "	" " "
	(+)	" " "	" " "	" " "	" " "
	(-)	" " "	" " "	" " "	" " "

Homatropine



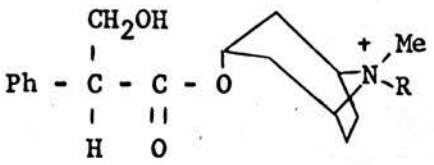
(±) R = H, Me, Et

R form R = H, Me, Et

S form R = H, Me, Et

Table 4

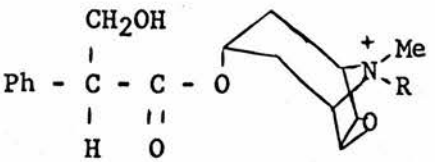
R and S hyoscyamine and derivatives



R isomer (R) = H, Me, Et, nPr, nBu.

S isomer (R) = H, Me, Et, nPr, nBu.

R and S hyoscine and derivatives



R isomer (R) = H, Me, Et, nPr, nBu.

S isomer (R) = H, Me, Et, nPr.

ii) Racemic cyclohexylphenylacetic acid was obtained from the hydrolysis of α-cyclohexylphenylacetonitrile, prepared as described by Hancock and Cope (1945), except that commercial sodamide was used in place of sodium in liquid ammonia and benzene was the solvent.

The nitrile (111 g) was hydrolysed by refluxing for 6 hrs with a ten-fold excess of 70% w/v sulphuric acid. The solid which formed on cooling was filtered off and washed thoroughly with water. This material (123 g) was recrystallised from methanol, yield (83 g; 69%), m.p. 153°.

Resolution of cyclohexylphenylacetic acid

Quinine and quinidine were used; the (+) acid gives a quinine salt which is less soluble in ethanol, the (-) acid forms a quinidine salt which is less soluble in ethyl acetate.

The racemic acid (170 g) was added to quinine (252 g) dissolved in boiling ethanol and the solution placed in the refrigerator for 2 hrs.

The salt which crystallised was filtered off, and after eight recrystallisations from ethanol had a constant melting point and rotation.

m.p. 170.8-171.4°

$[\alpha]_D^{25}$ +11.05° (c = 20, chloroform)

The mother-liquors, left after filtration of the quinine salt of the (+) acid, were combined and the solvent distilled off. The residue was treated with excess 2M sulphuric acid and extracted with ether. The extract was dried over magnesium sulphate and the solvent was distilled off to give the acid (85 g), which was slightly

levorotatory. This was added to quinidine (125 g) dissolved in boiling ethyl acetate. The hot solution was filtered and left to crystallise in the refrigerator overnight. The quinidine salt was filtered off and after four recrystallisations from ethyl acetate had constant melting point and rotation.

m.p. 133.4-134.4°

$[\alpha]_D^{25}$ +48.10° (c = 20, chloroform)

The rotations of the quinine and quinidine salts in chloroform were very temperature sensitive.

quinine salt $[\alpha]_D^{30}$ +5.50° (c = 20, chloroform)

With the quinidine salt the variation was of the same order i.e. 1° change in observed rotation per 5° alteration in temperature. Here however higher temperatures gave higher rotations.

All rotations were therefore measured in a cell which was surrounded by a water jacket connected to a thermostat at 25°C.

The resolved acids were obtained from the quinine and quinidine salts by treatment with excess 1N sulphuric acid and extraction into ether. The extracts were dried over magnesium sulphate and the solvent removed. The crude acids were recrystallised from 40-60° petroleum ether.

(+) acid (41 g; 48%) m.p. 100-102° $[\alpha]_D^{25}$ +38.7° (c = 20, chloroform)

(-) acid (33 g; 39%) m.p. 100-101° $[\alpha]_D^{25}$ -38.8° (c = 20, chloroform)

iii) Racemic cyclohexylphenylglycollic acid (hexahydrobenzilic acid) was prepared from ethylbenzoylformate (Corson, and others, 1928) and cyclohexylmagnesium bromide as described by Hoffmann and Schellenberg (1947), but with two slight modifications. After the cyclohexylmagnesium bromide had been added the solution was refluxed for 12 hrs, instead of being left to stand for 24 hrs at room temperature. The cold solution was treated with crushed ice and hydrochloric acid in the usual way. The ether extract was separated and washed with sodium bicarbonate. It was however then well shaken for 1½ hrs with a saturated sodium bisulphite solution. The ether layer was separated, washed with water, dried with magnesium sulphate and fractionally distilled.

cyclohexylphenylglycollic acid ethyl ester yield 53%

b.p. 146-150°/0.8 mm n_D^{25} 1.5152

The ester was hydrolysed as described by Hoffmann and Schellenberg and the resulting acid recrystallised from 50% aqueous ethanol.

Yield 80% m.p. 167.7-168.1°

(Hoffmann and Schellenberg gave m.p. 161-3°)

Resolution of cyclohexylphenylglycollic acid

This acid has been resolved using quinine (Patent, 1963) and with (+) and (-) amphetamine (Ellenbroek, 1964). The R and S forms have also been made by a stereospecific route (Inch, Ley and Rich, 1968) which shows that the (-) acid has the R configuration.

In this work quinine was used to obtain the S acid as described (Patent, 1963). After six recrystallisations from ethanol the salt had a constant melting point and rotation.

m.p. 222.4-222.6° dec.

$[\alpha]_D^{25}$ -76.0° (c = 4.0, chloroform)

It was found that the R acid formed the less soluble salt with (-) ephedrine in aqueous ethanol. Partially R acid from the mother-liquors of the S acid resolution, along with some racemic acid (total, 70 g), was dissolved in hot ethanol. (-)Ephedrine (52 g) dissolved in aqueous ethanol was added and the solution put in the refrigerator overnight. The solid which crystallised (62 g) was filtered off and after four recrystallisations from aqueous ethanol had a constant melting point and rotation.

m.p. 170.8-171.6°

$[\alpha]_D^{25}$ -43.5° (c = 2, ethanol)

The resolved acids were obtained by treating the salts with excess 2N hydrochloric acid, and shaking with ether. The extracts were dried over magnesium sulphate, the solvent distilled off and the residues recrystallised from 80-100° petroleum ether.

S acid (16 g; 50%) m.p. 140.6-141.1° $[\alpha]_D^{25}$ +24.8° (c = 4.5, ethanol)

R acid (19 g; 50%) m.p. 141.9-142.3° $[\alpha]_D^{25}$ -24.9° (c = 4.5, ethanol)

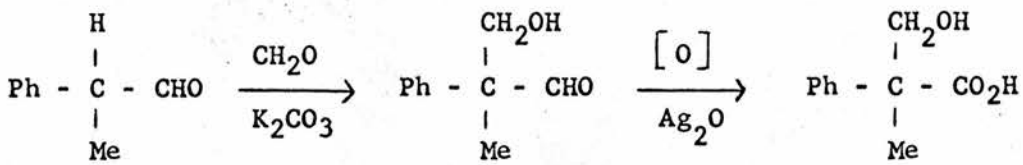
(Patent, 1963) gave

(+) acid m.p. 142-3° $[\alpha]_D$ +25.8 ± 1° (c = 4.483, ethanol)

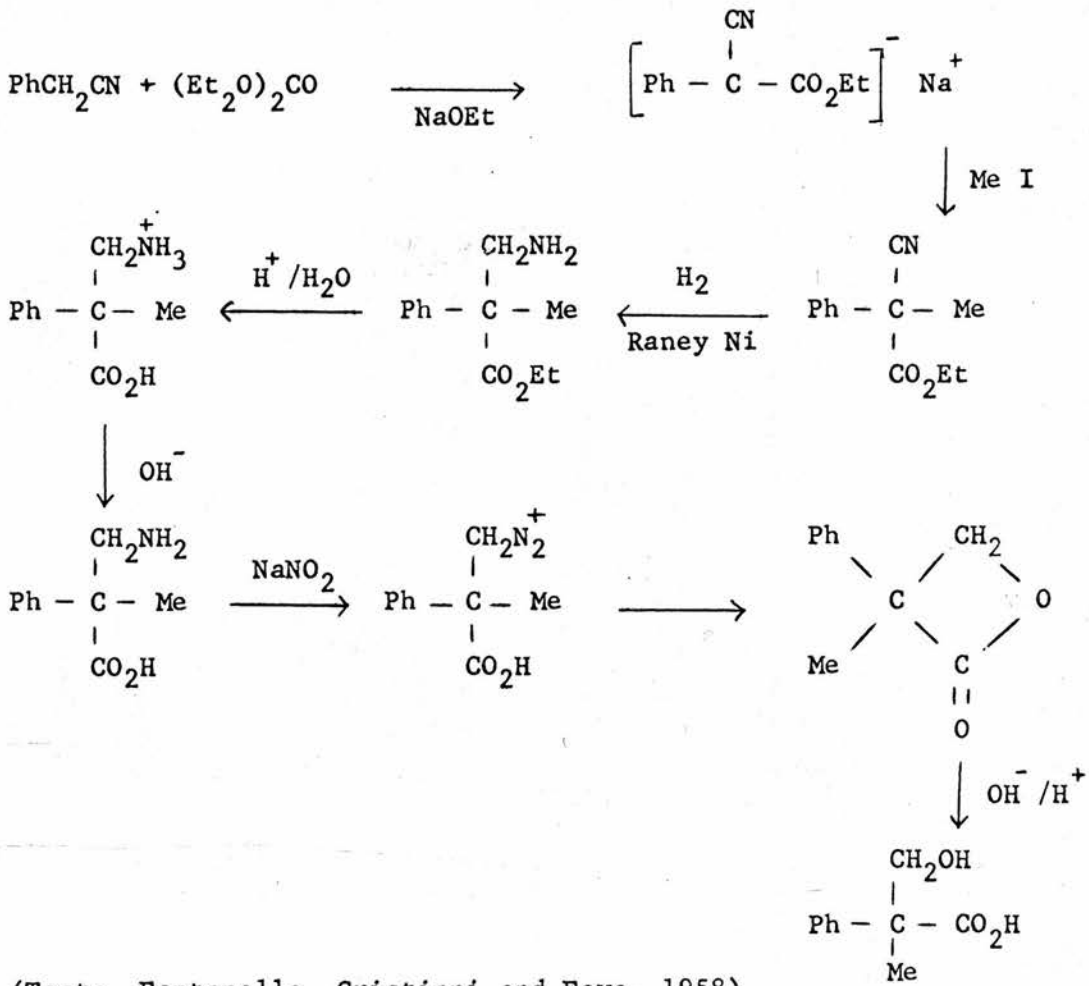
(-) acid m.p. 145° $[\alpha]_D$ -25.2 ± 1° (c = 4.483, ethanol)

iv) Racemic ∝-methyltropic acid

Three routes were attempted.

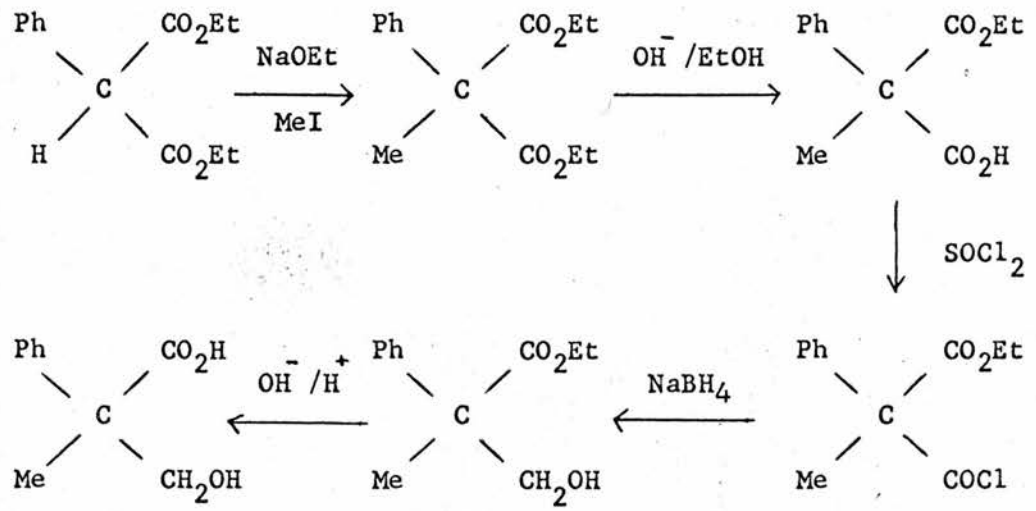
1. 

(Ellenbroek, 1964)

2. 

(Testa, Fontanella, Cristiani and Fava, 1958)

3.



(Vecchi and Melone, 1959)

The first route was not found to be convenient for the preparation of large quantities because of the very large volume of ethanol and water needed in the oxidation.

The second route was not used on a large scale because the diazotisation gave a mixture which when worked-up gave 2-benzyl-2-hydroxypropionic acid as well as the desired product.

The third route was used to make the racemic acid in quantity.

Diethyl(2-methyl-2-phenyl)malonate was prepared from diethyl (2-phenyl)malonate by a method similar to that described by Adams and Kamm (1925).

Yield 83% b.p. $100^{\circ}/0.3$ mm n_D^{25} 1.4915

This ester (230 g) was half-hydrolysed exactly as described by Vecchi and Melone (1959) and the crude product treated with thionyl chloride and distilled. The acid chloride (123 g; 56%) boiled at $102^{\circ}/0.3$ mm and had n_D^{25} 1.5117. It was reduced in approximately 50 g batches with sodium borohydride in anhydrous dioxan as described. The ethyl α-methyltropate obtained (74 g; 70%) had b.p. $100^{\circ}/0.3$ mm

n_D^{25} 1.5150. This was hydrolysed with 10% sodium hydroxide followed by acidification to give ∝-methyltropic acid in quantitative yield m.p. 89°. Vecchi and Melone gave m.p. 91-2° for material crystallised from benzene/petroleum ether.

Resolution of ∝-methyltropic acid

Because the (+) acid forms the less biologically active derivatives it must be thoroughly resolved and this is usually more easily achieved if the less soluble salt with an optically active base can be obtained first. Although this is the case with the brucine salt the yield of pure resolved acid is low and the base itself is highly toxic. It was decided to attempt resolution with other optically active bases. Because the (-) acid had been obtained as the quinine salt it was hoped that the (+) acid might be resolved using quinidine, but this salt could not be crystallised. However, a salt with (-) ephedrine did crystallise from ethyl acetate but this was of the (-) acid. At this point Professor G. Maffii of Lepetit SpA. very kindly provided large quantities of (+) and (-) ∝-methyltropic acid: (-)∝-methylytropic acid is used to make their drug Levopamate.

(+) ∝-methyltropic acid m.p. 86-7° $[\alpha]_D$ + 25.8°

(-) ∝-methyltropic acid m.p. 85-6° $[\alpha]_D$ -27.0°

Resolution of the bases

1) Hyoscyamine

The base was resolved, using camphor-10-sulphonic acid, almost exactly as described by Werner and Miltenberger, (1960). The salt with S-hyoscyamine crystallized first. This was not purified further because S-hyoscyamine can be obtained commercially. The mother liquors were concentrated, and the solid residues were extracted with hot ethyl acetate, (instead of acetone). This removed mostly the R-hyoscyamine salt which crystallised when the solution cooled, and which was subsequently recrystallized from acetone as in the original method.

(R)-hyoscyamine (+) camphor-10-sulphonate had m.p. 134-6°, $[\alpha]_D^{25}$ + 27.9° c = 5, water.

Werner and Miltenberger gave m.p. 135-6°, $[\alpha]_D^{20}$ +27.3° c = 0.094, water.

The salt was made alkaline with sodium carbonate and the free base was quickly extracted into chloroform and dried with magnesium sulphate. The base was recrystallized from 80°-100° petroleum ether.

(R)-hyoscyamine

m.p. 107.2-108.2° $[\alpha]_D^{26}$ + 21.4 c = 5, 50% aqueous ethanol.

Werner and Miltenberger gave

m.p. 105-6° $[\alpha]_D^{20}$ + 21° c = 0.173, 50% aqueous ethanol.

(S)-hyoscyamine hydrobromide (Macfarlane Smith) was converted into the free base as above.

(S)-hyoscyamine

m.p. 103.8-104.6° $[\alpha]_D^{23}$ -24.0 c = 5, 50% aqueous ethanol.

Werner and Miltenberger gave

m.p. 108-9° $[\alpha]_D^{20}$ -21° c = 0.173, 50% aqueous ethanol.

2) Hyoscine

Naturally occurring hyoscine has the (S) configuration. (S)-hyoscine was obtained as the hydrobromide, (B.D.H.).

To help avoid the risk of racemisation during aqueous alkaline extraction of the free base, and to prevent loss into the aqueous phase, the following procedure was used:-

(S)-Hyoscine hydrobromide $3H_2O$ (2 g) was dissolved in hot ethanol (10 ml) and triethylamine (0.5 g) was added. The solution was concentrated under vacuum to approximately 5 ml and ether (approx. 50 ml) was added. The triethylamine hydrobromide which precipitated was filtered off and the solution was concentrated once more under vacuum. More ether was added, and the solution was refiltered and dried with magnesium sulphate. The ether was distilled off, leaving the hyoscine (1.1 g) as a sticky syrup.

The quaternary derivatives were made in the same way as those of hyoscyamine.

A sample of R-hyoscine hydrobromide (1.1 g) was very kindly provided by Dr. R.P. Paton. This was converted to the free base as described for the S isomer and quaternised.

Formation of derivatives

Esters Four methods were used.

A) From acid + alcohol:

$$RCO_2H + R'OH \underset{\leftarrow}{\overset{H^+}{\rightleftharpoons}} RCO_2R' + H_2O$$

Because the alcohols contained a basic nitrogen atom the amount of acid added had to be large enough to form the salt and

also to catalyse the reaction. By passing hydrogen chloride through a syrupy mixture of acid and alcohol at 110° the equilibrium could be moved in favour of the ester because any water formed was carried over in the gas stream.

B) By transesterification:

$$RCO_2R'' + R'OH \underset{}{\overset{NaOR''}{\rightleftharpoons}} RCO_2R' + R''OH$$

The procedure employed was essentially that of Foster and Ing (1956) except that benzene was used as the solvent and the temperature was 80°.

This method was not suitable for acids which had a labile hydrogen atom attached to the asymmetric centre because racemisation occurred.

C) From acid chloride + alcohol:

$$RCOCl + R'OH \longrightarrow RCO_2R' + HCl$$

This method could not be used when the acid contained reactive groups e.g. hydroxyl. It was also found that if the acid contained a labile hydrogen atom attached to the asymmetric centre, the acid chloride racemised appreciably when it was distilled. The use of thionyl chloride which had not been specially purified also caused some racemisation even before the acid chloride was distilled.

The method is however otherwise very useful because the reaction is irreversible, and the hydrogen chloride which is formed reacts with the amino group to give the hydrochloride salt of the desired ester in almost quantitative yield.

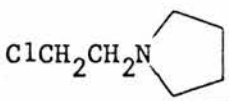
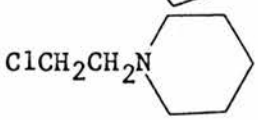
D) From N-substituted chloroalkylamines + the acid or its potassium salt:

i) $RCO_2H + Cl\,R' \xrightarrow{\text{i-Propanol}} RCO_2R' + HCl$

The method used was that described by Horenstein and Pählicke (1938) as modified by Burtner and Cusic (1943).

ii) $RCO_2^- K^+ + Cl\,R' \xrightarrow{\text{Acetone}} RCO_2R' + KCl$

A procedure similar to that described by Miescher and Hoffman (1941) was used. This was probably the best method for the synthesis of esters from amino alcohols and acids containing reactive groups. The conditions were very mild and racemisation was therefore less likely. The main problem was that some of the chloroalkylamines cyclised when heated or were left to stand. They were therefore stored as the hydrochloride and liberated just before use. The hydrochloride was dissolved in the minimum volume of water and shaken with excess 40% potassium hydroxide solution and ether. The ether extract was dried over magnesium sulphate and the solvent removed. All the chloroalkylamines except N,N-dimethyl-2-chloroethylamine were distilled under vacuum (water-pump). The dimethyl compound was low-boiling and it was found that it was best merely to remove most of the solvent and use the crude material.

	Appropriate B.P./8 mm	
$ClCH_2CH_2NEt_2$	50-55°	
	55-60°	(Tends to froth and is best distilled from a flask containing glass wool)
	65-70°	

The pyrrolidino and piperidino-2-chloroethylamine hydrochlorides were easily made from the corresponding alcohols. The alcohol was dissolved in chloroform and run slowly into a large excess of thionyl chloride in chloroform. The solvent and excess thionyl chloride were removed under vacuum to give the crude product which was used without purification.

Esters of tropine or scopine were not made by this route because it was not known whether the chlorine atom was in an axial or equatorial position and this means that the structure of the ester is uncertain.

The reaction also failed with some chloroalkyl amines e.g. N-methyl-4-chloropiperidine did not react with potassium benzilate even at 120° under pressure.

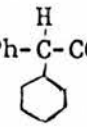
Examples of the methods

A) Racemic mandelic acid (4.0 g) and tropine (2.8 g) were heated in an oil-bath at 110° and hydrogen chloride was blown through the syrupy mixture for 6 hours. The reaction mixture was cooled and dissolved in a small volume of water. The solution was shaken four times with ether to remove unreacted acid. It was then made alkaline with potassium carbonate and extracted four times with ether. The combined ether extracts were dried over magnesium sulphate and the ether was distilled off leaving an oil (4.1 g). This could not be purified by distillation even at pressures as low as 0.001mm because it decomposed. TLC with silica gel and pyridine as solvent showed two spots when exposed to iodine vapour. The slow running spot (tropine) was very small and the crude product was used to make the quaternary derivatives without further purification.

B) Sodium (0.2 g) was dissolved in a solution of N,N-dimethylamino-ethanol (12 g) in sodium dried benzene (20 ml). Ethyl mandelate (9 g) was added and a small amount of solid formed. The mixture was distilled and further amounts of benzene were added from time to time, to keep the volume approximately constant. After about 5 hours ethanol could no longer be detected in the distillate by GLC. The benzene was removed under vacuum (water-pump) and the product extracted with 2 N hydrochloric acid. This extract was made alkaline with excess potassium carbonate, shaken with ether and the ether layer dried with magnesium sulphate. The ether was removed leaving an oil (5.5 g) which could not be distilled. TLC on silica gel using methanol as solvent showed three spots when exposed to iodine vapour, the centre one being the main component. The U.V. spectra showed that the material in the two faster running spots had aromatic groups present. Large scale column chromatography using silica gel with methanol as solvent caused almost quantitative conversion of the amino esters to methyl mandelate and free amino alcohol. The crude reaction products were therefore converted directly to the quaternary derivatives which were purified by recrystallisation.

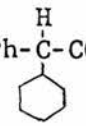
C) (+) cyclohexylphenylacetyl chloride (4.0 g) was treated dropwise with freshly distilled N,N-diethylaminoethanol (5.0 g). Heat was evolved and a solid formed. The reaction mixture was left overnight and then dissolved in the minimum of water. The aqueous solution was made alkaline with potassium carbonate and shaken with ether. The ether extract was dried over magnesium sulphate and the solvent removed. The residual oil was distilled under reduced pressure, first with a water pump which removed unreacted amino-alcohol, then

the remainder was fractionated at approximately 0.1 mm

(+)  b.p. 144-5°/0.1 mm. Yield 4.4 g, 82%

It was found that the product was partially racemised so this method could not be used to prepare esters of the resolved acids.

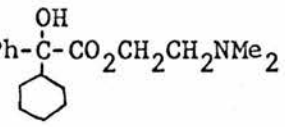
D) i) (+) cyclohexylphenylacetic acid (3.0 g) was dissolved in hot i-propanol (25 ml) and N,N-diethyl-2-chloroethylamine (2.2 g) was added. The solution was refluxed overnight and the solvent was distilled off under vacuum. Ether was added and the solid hydrochloride filtered off (4.7 g). This was dissolved in the minimum of water, the solution was made alkaline with potassium carbonate and shaken with ether. The ether extract was dried over magnesium sulphate and the ether distilled off. The resulting oil was distilled to give

(+)  B.p. 140-150°/0.002-0.005 mm. Yield 3.0 g, 69%

For the preparation of mandelic esters the solution was refluxed for 2 hrs, filtered and anhydrous ether added till there was just a permanent turbidity. The solution was left in the refrigerator overnight and the hydrochloride which crystallised was filtered off and recrystallised from a mixture of i-propanol and ethyl acetate. The free base obtained from the hydrochloride could not be distilled (see page 22).

ii) S-cyclohexylphenyl glycollic acid (3.5 g) was dissolved in acetone (50 ml). Potassium carbonate (1.03 g) was added and the solution was

warmed; carbon dioxide was evolved. When most of the potassium carbonate had gone into solution, N,N-dimethyl-2-chloroethylamine (5.5 g) was added and the solution refluxed overnight. When cold the solution was filtered and the acetone distilled off. Ether was added, the solution was refiltered and the solvent removed. The resulting oil was distilled



S Ph-C(OH)-$CO_2CH_2CH_2NMe_2$ B.p. 150-2°/0.002 mm. Yield 3.0 g, 66%

It was found with the α-methyltropic esters that all the reactants could be added simultaneously without affecting the yield.

Hydrochloride salts of the amino esters

These were made by bubbling hydrogen chloride through an ethanolic solution of the base and distilling off the solvent under vacuum. More ethanol was then added and the solvent removed once more. This was repeated a few times to remove any water which might be present. The dry hydrochlorides were then recrystallised from a suitable solvent.

The methods by which the esters were made, and their boiling points are shown in Table 5.

Table 5

Methods of preparing esters

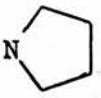
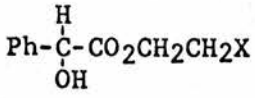
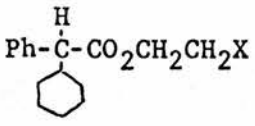
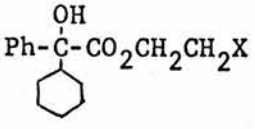
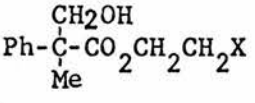
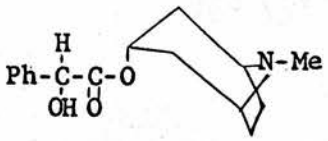
	X =	NMe_2	NEt_2		
					
(±)		B	Di	Di	Di
R, S		-	Di	Di	Di
					
(±)		Already made by method C			
+, -		Di	Di	Di	Di
					
(±)		Already made by method Dii			
R, S		Dii	Dii	Dii	Dii
					
(±)					
+, -		Dii	Dii	Dii	Dii

Table 5 (cont'd)

(Approximate yields A, B 50-60% C 80-90% Di, Dii 60-70%)

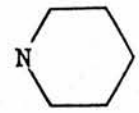
(±)  methods A and B

R " method A

S

Table 5 (cont'd)

B.P. (mm) and n_D^{25} for esters

	X = NMe_2	NEt_2		
Ph-C(H)(OH)-$CO_2CH_2CH_2X$				
(±)				
R	Could not be distilled			
S				
Ph-C(H)()-$CO_2CH_2CH_2X$				
(±)		Previously made		
(+)	120-8° (5×10^{-4})	140-50° (2×10^{-3})	160-2° (2×10^{-3})	174-5° (3×10^{-3})
(-)	125-8° (10^{-3})	148-50° (10^{-3})	164-6° (6×10^{-3})	174-8° (4×10^{-3})
Ph-C(OH)()-$CO_2CH_2CH_2X$				
(±)		Previously made		
R	152-4° (2×10^{-3})	157-9° (5×10^{-3})	160-8° (8×10^{-4})	170-4° (10^{-3})
S	150-2° (2×10^{-3}) 1.5170	158-9° (10^{-3})	178° (4×10^{-3})	Not distilled
Ph-C(CH_2OH)(Me)-$CO_2CH_2CH_2X$				
(±)	100-8° (2×10^{-3}) 1.5133	Not distilled	110-5° (4×10^{-3}) 1.5248	122-140 ($2\text{-}4\times10^{-3}$) 1.5261
(+)	120-8° (6×10^{-3}) 1.5131	120° (4×10^{-3})	110° (2×10^{-4})	140-2° (4×10^{-4})
(-)	122-8° (6×10^{-3})	123-5° (4×10^{-3})	116° (2×10^{-4})	140-2° (4×10^{-4})

Quaternary salts

The tertiary bases were usually dissolved in ethyl methyl ketone and treated with an excess of methyl or ethyl iodide and the solution left for two days. If no crystals had formed the solution was refluxed for approximately 2 hrs and ethyl acetate was added until there was just a permanent turbidity. The solution was put in the refrigerator and was scratched from time to time until crystals formed. These were then filtered off and recrystallised.

With hyoscine and hyoscyamine, acetonitrile was used as solvent because it has a high dielectric constant and has been used for the preparation of buscopan (hyoscine n-butyl bromide) (Patent, 1954). For alkylation with groups larger than methyl it was necessary to heat the mixture under reflux for approximately 12-18 hrs.

Melting points and analyses for the quaternary compounds and the salts of the tertiary bases are given in tables 6-12
The quaternary salts are iodides unless otherwise stated. The tertiary salts are hydrochlorides unless otherwise stated.

Rotations of the optically active forms are given as molar rotations at the wavelengths shown. The solvent and concentration are indicated, and the cell path length was almost always 5 cm.

Recrystallisation

Most recrystallisations were from ethyl methyl ketone alone, but ethanol or ethyl acetate were added if necessary.

Table 6

Melting points and analyses of (±) Ph-C(OH)H-$CO_2CH_2CH_2R$

Onium group (R)	Melting point (°C)	% Halide Theory	% Halide Found
$^{+}NMe_3$	112-116	34.73	34.57
$^{+}NMe_2Et$	85-88	33.47	33.33
$^{+}NEt_2Me$	122-123	32.27	32.28
$^{+}NEt_3$	105-106	31.16	31.12
	103-106	32.48	32.58
	86-89	26.92	26.87
	89-93	31.31	31.20
	99-101	30.26	30.26
Racemic homatropine			
$Me\ Br^{-}$	179-183	21.58	21.71
$Et\ Br^{-}$	215-220 dec	20.80	20.66

Table 6 (cont'd)

Melting points and analyses of R and S Ph-C(OH)H-$CO_2CH_2CH_2R$

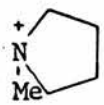
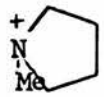
Onium group (R)	Melting point (°C) S	Melting point (°C) R	% Halide Theory	% Halide Found S	% Halide Found R
$^{+}NEt_2Me$	92-97	92-98	32.27	32.42	32.39
$^{+}NEt_3$	121-124	118-121	31.16	31.54	31.39
	107-109	107-109	32.43	32.25	32.22
	96-99	96-100	31.31	31.14	31.10
	109-111	104-108	31.31	31.32	31.25
	110-115	110-115	30.26	30.22	30.28

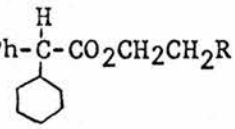
Table 6 (cont'd)

Rotations of R and S Ph-C(H)(OH)-$CO_2CH_2CH_2R$

Onium group	Molar rotation in degrees			
	589 nm		300 nm	
(R)	S	R	S	R
$^+NEt_2Me$	159	-157	1416	-1437
$^+NEt_3$	154	-151	1347	-1321
	166	-161	1471	-1414
	161	-158	1377	-1376
	174	-163	1535	-1421
	162	-161	1356	-1396

Concentration = 2 x 10^{-2} M/EtOH, Cell path length = 5 cm.

Table 7

Melting points and analyses of (+) and (-) 

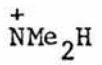
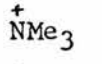
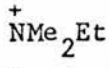
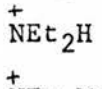
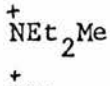
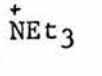
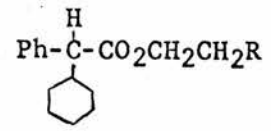
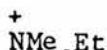
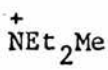
Onium group (R)	Melting point °C		% Halide Theory	Found	
	(+)	(-)		(+)	(-)
	138.8-139.6	138.6-139.8	10.81	11.02	11.00
	160.2-161.4	161.0-161.7	29.42	29.43	29.44
	125.2-126.0	124.6-125.4	28.49	28.48	28.63
	146.6-147.2	146.0-147.0	10.02	10.02	9.99
	111.0-111.7	111.0-111.7	27.62	27.74	27.57
	153.6-154.0	153.9-154.3	26.80	26.97	27.15
	153.6-154.6	154.9-155.5	10.07	10.15	10.22
	87.2-88.2	88.2-90.2	27.74	28.23	27.97
	127.2-128.3	129.0-129.6	26.92	27.38	27.19
	195.7-196.4	196.1-196.8	9.87	9.42	9.80
	146.4-148.0	146.5-148.0	26.92	26.42	26.84
	124.0-125.2	124.6-125.6	26.14	26.34	26.16

Table 7 (cont'd)

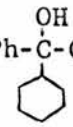
Rotations of (+) and (-) 

Onium group (R)	Molar rotation in degrees			
	589 nm		300 nm	
	(+)	(-)	(+)	(-)
	102	100	986	981
	121	120	1137	1128
	114	114	1115	1122
	96	98	1006	1009
	105	105	1081	1070
	99	97	1038	1047
	-	-	-	-
	104	103	1062	1066
	101	102	1052	1060
	106	105	1098	1074
	101	101	1074	1075
	93	94	1013	1030

Concentration = 5×10^{-2} M/EtOH. Cell path length = 5 cm.

Table 8

Melting points and analyses of R and S Ph-C(OH)-$CO_2CH_2CH_2R$



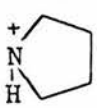
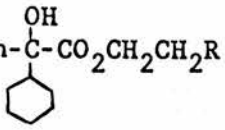
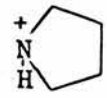
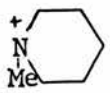
Onium group (R)	Melting point °C		% Halide Theory	% Halide Found	
	S	R		S	R
$^+NMe_2H$	197.0-197.4	197.4-197.7	10.37	10.45	10.52
$^+NMe_3$	141.1-142.3	141.6-142.4	28.36	28.05	28.18
$^+NMe_2Et$	165.2-165.8	165.0-165.9	27.51	27.53	27.55
$^+NEt_2H$	221.2-221.5	220.4-220.6	9.58	9.72	9.56
$^+NEt_2Me$	153.1-153.6	152.6-153.1	26.69	26.84	26.72
$^+NEt_3$	197.2-197.4 (dec)	196.5-196.7 (dec)	25.93	25.94	25.84
	207.2-207.4	206.3-206.5	9.64	9.76	9.68
	192.2-192.8	182.3-183.1	26.80	26.53	26.68
	140.0-140.8	153.4-154.2 sinters 140°	26.04	25.85	25.98
	224.2-224.4 (dec)	223.4-223.6 (dec)	9.29	9.43	9.32
	186.5-187.4	186.9-187.3	26.04	25.86	26.02
	172.2-173.2	172.2-173.2	25.31	25.47	25.23

Table 8 (cont'd)

Rotations of R and S 

Onium group	Molar rotation in degrees			
	589 nm		300 nm	
(R)	S	R	S	R
$^{+}NMe_2H$	-20	17	26	-28
$^{+}NMe_3$	-23	24	148	-150
$^{+}NMe_2Et$	-26	25	135	-132
$^{+}NEt_2H$	-14	13	43	-45
$^{+}NEt_2Me$	-26	26	107	-104
$^{+}NEt_3$	-29	28	66	-66
	-17	17	33	-31
	-25	26	123	-117
	-26	27	83	-78
	-17	15	78	-76
	-28	27	119	-117
	-26	25	93	-100

Concentration = 5×10^{-2}/MeOH Cell path length = 5 cm.

Table 9

Melting points and analyses of (±) Ph-C(CH$_2$OH)(Me)-CO$_2$CH$_2$CH$_2$R

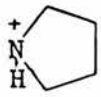
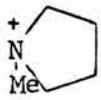
Onium group (R)	Melting point (°C)	% Halide Theory	% Halide Found
$^+NMe_2H$	78.1-80.1	12.32	12.57
$^+NMe_3$	128.0-129.3	32.27	31.99
$^+NMe_2Et$	114.8-115.8	31.16	31.00
$^+NEt_2H$	115.5-117.5	11.23	11.48
$^+NEt_2Me$	93.6-95.6	30.11	30.22
$^+NEt_3$	155.7-156.3	29.14	29.17
	74.4-76.1	11.30	11.36
	93.0-94.7	30.26	29.89
 Br^-	111.0-112.5	20.67	20.59
	132.0-134.8	10.81	10.85
	137.3-138.7	29.28	29.26
	97.0-98.3	28.36	28.18

Table 9 (cont'd)

Melting points and analyses of (+) and (-) Ph-$\overset{CH_2OH}{\overset{|}{C}}$(Me)-$CO_2CH_2CH_2R$

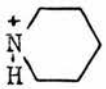
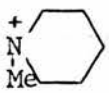
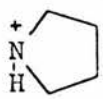
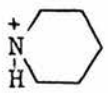
Onium group (R)	Melting point °C		% Halide Theory	% Halide Found	
	(+)	(-)		(+)	(-)
$^+NMe_2H$	77.5-79.0	76.2-80.0	12.32	12.40	12.40
$^+NMe_3$	116.4-117.9	117.1-118.4	32.27	32.18	32.02
$^+NMe_2Et$	131.0-131.7	128.2-130.2	31.16	31.19	31.15
$^+NEt_2H$	92.2-93.7	93.5-95.5	11.23	11.31	11.41
$^+NEt_2Me$	124.8-127.4	125.5-127.5	30.11	29.77	30.06
$^+NEt_3$	171.2-172.9	172.1-173.4	29.14	28.94	29.17
	89.0-90.6	85.0-87.5	11.30	11.28	11.31
	99.0-100.5	97.9-99.4	30.26	30.52	29.97
 Br^-	Could not be crystallised				
	141.8-143.0	126.0-126.8	10.81	11.01	11.00
	144.5-146.3	143.5-145.5	29.28	29.43	29.63
	102.0-103.0	102.0-103.3	28.36	28.28	28.48

Table 9 (cont'd)

Rotations of (+) and (-) Ph-C(Me)(CH$_2$OH)-CO$_2$CH$_2$CH$_2$R

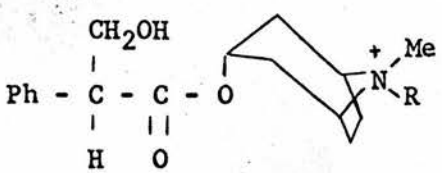
Onium group (R)		Molar rotation in degrees 589 nm (+)	589 nm (-)	300 nm (+)	300 nm (-)
$^+NMe_2H$		27.6	30.0	247	247
$^+NMe_3$		4.8	4.8	32.4	36.4
$^+NMe_2Et$		36.0	35.2	316	302
$^+NEt_2H$		31.2	31.2	262	256
$^+NEt_2Me$		38.0	38.8	332	328
$^+NEt_3$		43.2	44.4	358	360
		29.2	30.0	254	256
		32.0	34.4	296	291
	Br^-	34.4	35.2	304	289
		26.8	27.2	242	233
		33.2	34.0	294	289
		37.6	38.8	328	331

Concentration = 5 x 10^{-2}M/water. Cell path length = 5 cm.
for R = NMe_3 = 5 x 10^{-2}M/MeOH.

Ellenbroek and others (1965) give $[\alpha]_D$ +1.8 and -2.2 (cf +1.2 and -1.2 found here) for R = $\overset{+}{N}Me_3$ in methanol.

Table 10

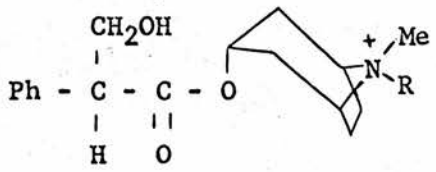
Melting points and analyses of R and S hyoscyamine and derivatives



(R)	Melting Point °C		% Halide Theory	% Halide Found	
	S	R		S	R
H Br^-	148.6-151.9	150.4-151.4	21.58	-	21.38
Me	186.1-186.6	185.5-185.9	29.43	29.19	29.13
Et	152.2-153.3	161.8-163.2	28.49	28.44	28.68
nPr	173.2-175.8	168.0-170.0	27.63	27.36	27.73
nBu	152.6-155.6	147.0-150.0	26.80	26.83	26.46

Table 10 (cont'd)

Rotations of R and S hyoscyamine and derivatives



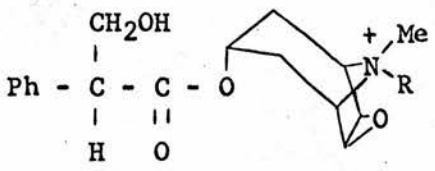
(R)	Molar rotation in degrees			
	589 nm		300 nm	
	S	R	S	R
H Br^-	-96	97	-991	995
Me	-93	94	-979	994
Et	-89	88	-946	912
nPr	-79	81	-877	869
nBu	-79	80	-887	861

Concentration = 4×10^{-2} M in water

Cell path length = 5 cm.

Table 11

Melting points and analyses of R and S hyoscine and derivatives



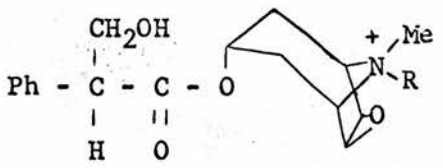
(R)	Melting Point °C		% Halide		
			Theory	Found	
	S	R		S	R
H Br^-	195.1-195.5	170-190	-	-	-
Me	220.7-221.0 (dec)[1]	210.0-210.9 (dec)	28.49	28.30	-
Et	185.7-186.0 (dec)[2]	182.0-182.8 (dec)	27.64	27.62	-
nPr	160.3-160.8 (dec)	157.1-157.4 (dec)	26.80	26.68	-
nBu	86.0-93.0	-	26.04	25.84	-

1 Schmidt (1894) gave m.p. 215°

2 Ibid, m.p. 186°

Table 11 (cont'd)

Rotations of R and S hyoscine and derivatives



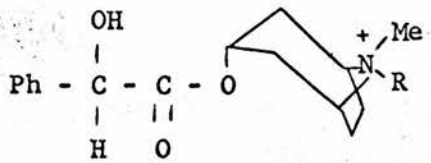
(R)	Molar rotation in degrees			
	589 nm		300 nm	
	S	R	S	R
H Br^-	-114	186	-1190	1218
Me	-109	103	-1184	1105
Et	-108	104	-1179	1147
nPr	-103	99	-1137	1107
nBu	-96	-	-1078	-

Concentration = 2×10^{-2} M in water

Cell path length = 5 cm

Table 12

Melting points and analyses of R and S homatropine and derivatives*



(R)	Melting point °C		% Halide		
			Theory	Found	
	S	R		S	R
H (sulphate)	232.7-232.8(b)[1]	225.7-225.9(a)[2]	-	-	-
Me	202-215(a)	213-216(a)	30.42	31.66(a)	31.42(a)
	210-214(b)	216-220(b)		33.16(b)	30.50(b)
Et	200-202(b)	202-204(a)	29.43	29.33(b)	29.47(a)

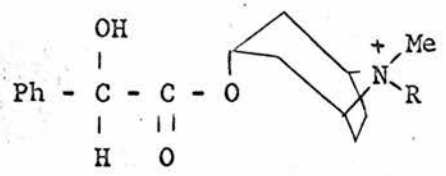
[1] Werner and Miltenberger gave m.p. 213°

[2] Ibid, m.p. 210°

* see footnote to following table

Table 12 (cont'd)

Rotations of R and S homatropine and derivatives



(R)	Molar rotation in degrees 589 nm		300 nm	
	S	R	S	R
H (sulphate)	404(b)	-417(a)	-	-
Me	149(a)	-175(a)	-	-1719(a)
	154(b)	-175(b)	-	-1737(b)
Et	175(b)	-191(a)	1653(b)	-1888(a)

Concentrations approximately 10^{-2} M in water

Cell path length = 5 cm

* R and S homatropine

Esterification of R and S mandelic acid with tropine by method A gave crude products which had rotations less than those quoted by Werner and Miltenberger (1960). TLC on silica gel using pyridine as solvent and developed with iodine vapour, indicated that the crude products were probably contaminated with tropine. To check that this and not racemisation was the cause of the low rotations the reaction products were divided into two portions. One was used directly to make the sulphate and quaternary derivatives (marked (a) in the tables) and the other was converted to the (+) camphor-10-sulphonates, which crystallised from acetone/ether and had melting points and rotations as described by Werner and Miltenberger, as had

Table 12 (cont'd)

the free bases regenerated from them. The derivatives from these purified samples are marked (b) in the tables. It appeared that racemisation did not take place during synthesis but the crude product obtained was badly contaminated with unreacted tropine.

Measurement of affinity constants

a) Isolated guinea-pig ileum

The method used was that of Edinburgh Staff (1968). A piece of guinea-pig ileum 2-3 cm long was suspended in aerated Tyrode solution at 37°C, containing 2.76×10^{-4}M hexamethonium. Two concentrations of carbachol, each in contact with the ileum for 30 sec, were applied alternately every 90 sec and the contractions were recorded with a lever attached to the core of a differential transformer connected to an oscillator and a pen-recorder. When the responses were steady, the Tyrode was replaced with Tyrode containing a known concentration of antagonist. At the same time the agonist solutions were replaced by others containing a much higher concentration of carbachol together with the same concentration of antagonist as was present in the wash Tyrode. The new carbachol concentrations were chosen in the expectation that the contractions in the presence of the antagonist would be approximately equal in size to those produced initially. The antagonist was assumed to be in equilibrium with the receptors when these contractions had reached a steady level. An automated apparatus similar in principle to that described by Schild (1947) was used to apply the drug solutions. Usually the average response to about six applications of each concentration of agonist was taken and because those in the presence of the antagonist did not exactly match the corresponding responses before it was applied, a correction was made using the shape of the log dose-response curve as in a four point assay. Every antagonist was tested in at least two concentrations in order to check that it was acting competitively. Usually the

concentrations were chosen so that the dose-ratios lay in the range 10-1000. When the affinity constants derived from these values agreed, indicating competition, the average value was taken. All estimates used in the calculation of the overall mean were based on results obtained with separate pieces of ileum (usually six).

Hexamethonium was used throughout as a ganglion blocking agent largely because many affinity constants had already been measured in its presence and this allowed direct comparison with these earlier results. It had been used in this earlier work because some of the compounds whose affinities had been measured were partial agonists and might have had some ganglion stimulant action. In addition it was possible that the high concentrations of carbachol used in the experiments might have affected the preparation by actions at ganglia, perhaps involving circular muscle. Hexamethonium does in fact have a slight blocking action at the postganglionic parasympathetic receptors and has been found to affect estimates of log K obtained with the bronchial strip preparation. In a comparison of estimates of log K for twelve compounds on the ileum, however, hexamethonium had no significant effect (Barlow, Franks and Pearson 1972b).

b) Isolated guinea-pig bronchial strip and iris

Experiments, similar to those described above, were made with the bronchial strip and iris preparations.

The bronchial strip was bathed in Krebs solution, gassed with oxygen plus five per cent carbon dioxide, at 37^{o} and the responses were recorded isometrically. The agonist, carbachol, was allowed to act for 5 min and the interval between doses was 15 min.

The iris was also bathed with Krebs solution, gassed with oxygen plus five per cent carbon dioxide, at 37° and the responses were recorded with a light beam and photocell connected to a potentiometric recorder. The agonist, carbachol, was allowed to act for 1 min and the interval between doses was 10 min.

Full details of these preparations are described by Barlow, Franks and Pearson (1972b).

Errors

An idea of the errors in the estimates of log K is given by the standard error and number of estimates. Some compounds, however, have been tested at different times and by different people and it seems that the statistical error within one group of measurements is probably an underestimate. In fact it seems probable that values which differ by less than 0.1 log units should not be considered to be different from each other.

RESULTS

Results

The results obtained on the isolated guinea-pig ileum are shown in tables 13-19. They are expressed as the mean value of log K, $\pm$ the standard error, with the number of estimates in parenthesis.

Some pairs of enantiomers were also tested on the bronchial strip and isolated iris preparations and the results are shown in table 20.

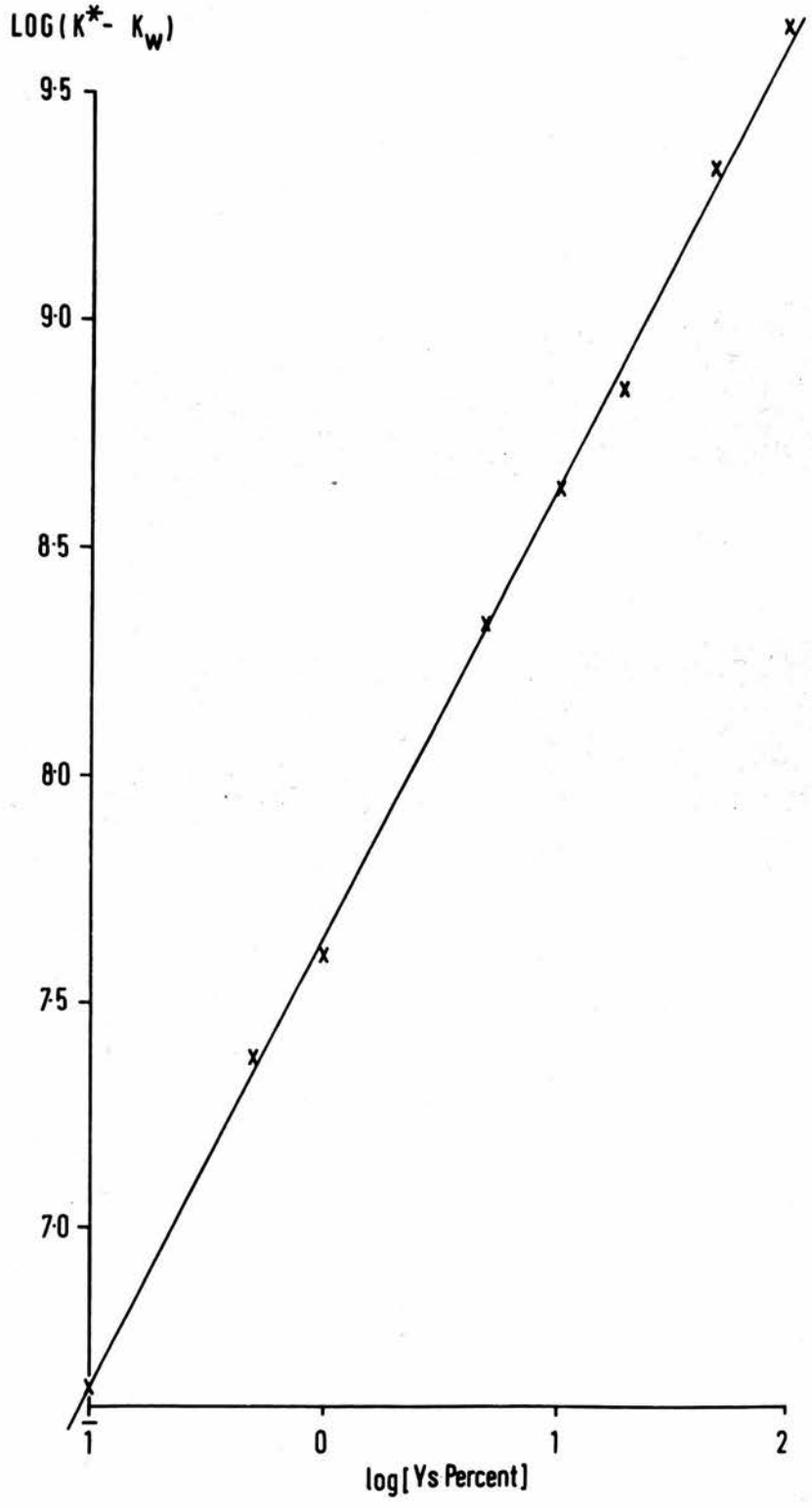
In addition, experiments were made to study the effect of enantiomeric composition on the experimentally observed affinity constants and the results are shown in table 21. If the enantiomers are both competing for the same receptors as the agonist, the apparent affinity constant K* should be related to that of the weaker, K_w, and that of the stronger, K_s, by the expression

$$K^* - K_w = y_s(K_s - K_w)$$

where y_s is the proportion of stronger isomer present in a mixture. Figure 1 shows the graph of log $(K^* - K_w)$ plotted against log y_s and supports the idea that the compounds are acting competitively. Similar results were obtained in experiments made with the enantiomeric forms of benzhexol and were shown to be quite different from what would be expected if either isomer acted non-competitively (Barlow, Franks and Pearson, 1972a). Accordingly it is possible to calculate values of log K for the racemate ($y = \frac{1}{2}$) and compare them with values obtained from the racemate itself. This can be used to obtain some idea of the reproducibility of estimates of log K.

Figure 1

The logarithm of the enantiomeric composition (log Y_S%) is plotted against the logarithm of the difference between the observed affinity constant K* and the affinity constant of the weaker isomer K_w. Actual values are shown in table 21.



Further the degree of resolution should be related to the stereospecific index, S ($= K_s/K_w$) by the expression

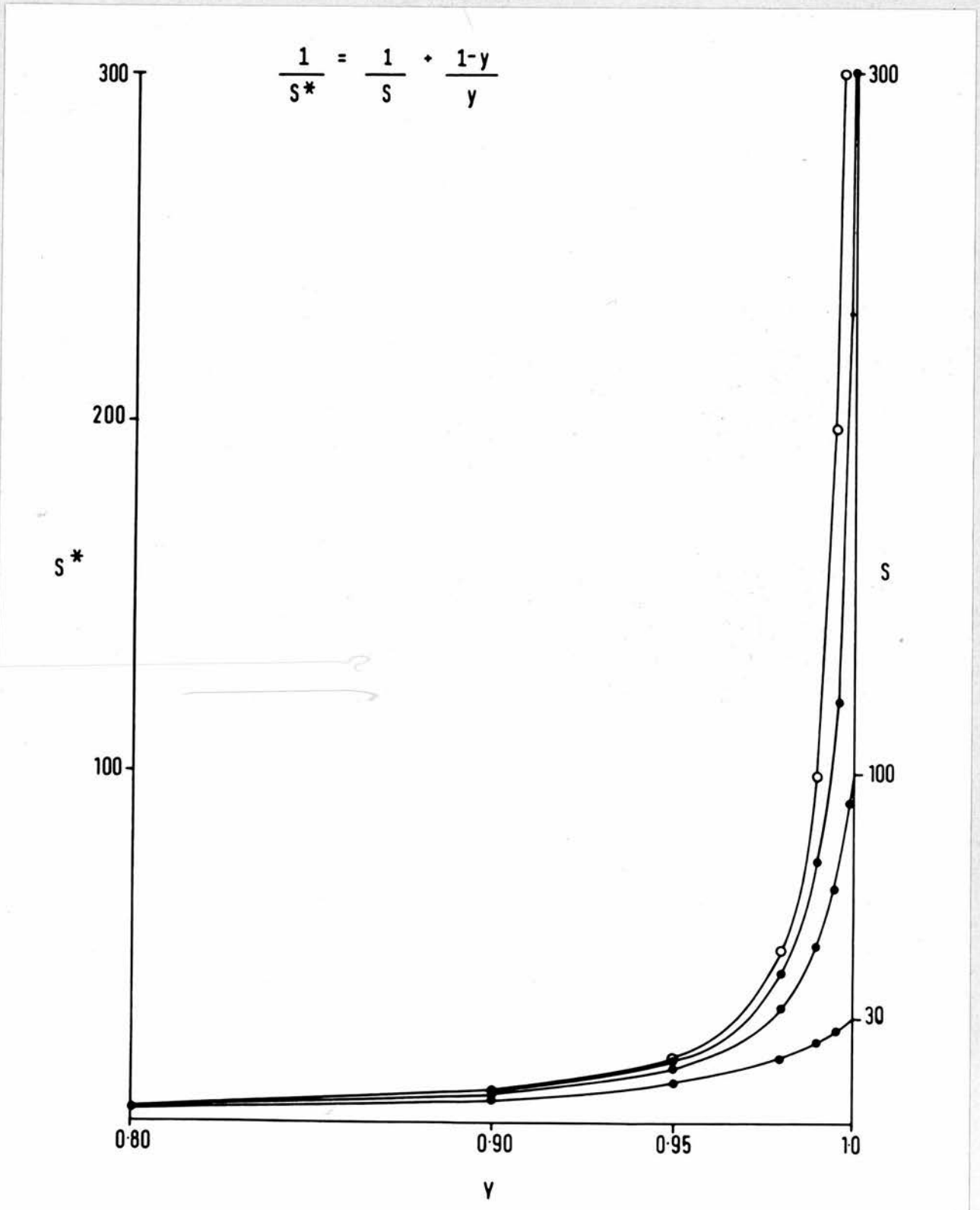
$$\frac{I}{S^*} = \frac{I}{S} + \frac{I-y}{y}$$

where y is the proportion of an enantiomer present in the sample and it is assumed that the enantiomers have rotations of equal and opposite signs so are equally (probably incompletely) resolved. S* is the apparent stereospecific index obtained from K_s^*/K_w^* where K_s^* and K_w^* are the experimentally measured values of the affinity constant. This expression, illustrated in Figure 2, therefore makes it possible to set limits to the degree of resolution which has been achieved. For example, if the true stereospecific index was 30 then the observed value for 99% resolution would be 23, for 98% resolution it would be 18 and for 95% resolution it would be 11. If it were assumed that the true stereospecific index was infinite, i.e. one isomer was totally inactive, then an observed value of 100 could only be achieved if resolution was 99.1% complete. Because it is unlikely that one isomer will have no affinity for the receptors, the true degree of resolution must be regarded as being better than this. Using this relation it was possible to determine the minimum degree of resolution in the series of enantiomers made.

The values of log K shown in tables 13-19 have been used to obtain values of the log of the stereospecific index together with the 95% confidence limits, which are shown in tables 22-27, together with the estimates of the values of log K for racemic mixtures which are compared with the experimental values. Usually the predicted values for the racemate are close to those observed;

Figure 2

Effect of enantiomeric composition (y) on the observed stereospecific index S*. The true stereospecific index S is the value obtained when y = 1.

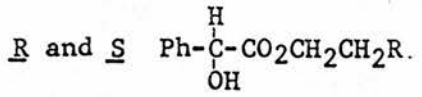


in eight instances out of 25 the discrepancy exceeded 0.1 log units but in no instance did it exceed 0.2 log units.

Table 13

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.

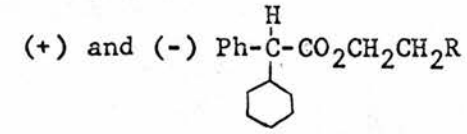


Onium group (R)	S	R
$\overset{+}{N}Me_3$	—	—
$\overset{+}{N}Me_2Et$	—	—
$\overset{+}{N}Et_2Me$	6.044 ± 0.030(12)	6.153 ± 0.055(7)
$\overset{+}{N}Et_3$	6.117 ± 0.022(8)	6.103 ± 0.097(9)
	5.597 ± 0.031(7)	5.974 ± 0.014(5)
	5.911 ± 0.036(7)	6.368 ± 0.025(8)
	5.321 ± 0.039(5)	5.674 ± 0.009(5)
	5.501 ± 0.01(7)	6.021 ± 0.065(6)

Table 14

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.

(+) and (-) 

Onium group

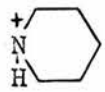
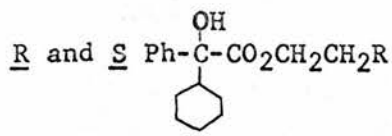
(R)	(+)	(-)
$^{+}NMe_2H$	7.392 ± 0.040(8)	7.952 ± 0.064(6)
$^{+}NEt_2H$	8.000 ± 0.020(6)	8.216 ± 0.043(6)
	7.881 ± 0.032(6)	8.262 ± 0.020(6)
	8.427 ± 0.021(6)	8.115 ± 0.019(8)
$^{+}NMe_3$	8.100 ± 0.033(6)	8.574 ± 0.010(8)
$^{+}NMe_2Et$	8.500 ± 0.038(8)	8.992 ± 0.024(6)
$^{+}NMeEt_2$	8.490 ± 0.032(6)	8.849 ± 0.049(7)
$^{+}NEt_3$	8.431 ± 0.016(4)	8.533 ± 0.016(7)
	8.301 ± 0.023(7)	8.675 ± 0.027(6)
	8.568 ± 0.020(6)	8.935 ± 0.025(7)
	8.301 ± 0.016(6)	8.303 ± 0.013(7)
	8.224 ± 0.050(7)	8.151 ± 0.034(6)

Table 15

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.



Onium group

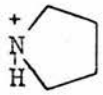
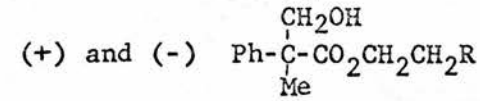
(R)	S	R
$\overset{+}{N}Me_2H$	6.977 ± 0.046(7)	8.866 ± 0.067(11)
$\overset{+}{N}Et_2H$	7.756 ± 0.053(7)	9.299 ± 0.054(5)
	7.191 ± 0.025(6)	8.860 ± 0.056(10)
	6.935 ± 0.048(8)	9.008 ± 0.071(9)
$\overset{+}{N}Me_3$	7.257 ± 0.021(13)	9.647 ± 0.073(11)
$\overset{+}{N}Me_2Et$	7.882 ± 0.019(7)	10.040 ± 0.039(8)
$\overset{+}{N}MeEt_2$	8.150 ± 0.036(5)	10.000 ± 0.039(7)
$\overset{+}{N}Et_3$	7.989 ± 0.035(7)	9.600 ± 0.068(8)
	7.488 ± 0.046(6)	9.635 ± 0.047(6)
	7.765 ± 0.059(8)	9.723 ± 0.024(5)
	7.334 ± 0.033(6)	9.474 ± 0.041(6)
	7.413 ± 0.013(6)	9.253 ± 0.037(8)

Table 16

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.

(+) and (-) 

Onium group

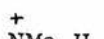
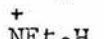
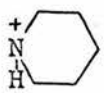
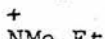
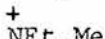
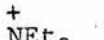
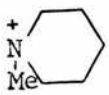
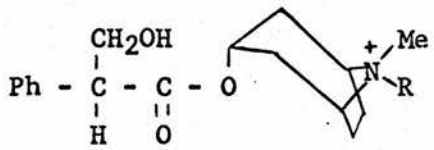
(R)	(+)	(-)
	5.570 ± 0.031(5)	7.354 ± 0.003(6)
	6.126 ± 0.050(7)	7.996 ± 0.016(5)
	5.126 ± 0.039(5)	7.645 ± 0.038(6)
	5.312 ± 0.037(6)	7.452 ± 0.026(7)
	6.058 ± 0.018(7)	8.119 ± 0.035(8)
	6.648 ± 0.061(6)	8.841 ± 0.031(8)
	6.681 ± 0.030(7)	8.478 ± 0.037(7)
	6.607 ± 0.031(7)	8.286 ± 0.016(8)
	6.564 ± 0.006(5)	8.229 ± 0.026(7)
	6.739 ± 0.035(7)	8.289 ± 0.020(6)
	6.406 ± 0.022(5)	8.099 ± 0.037(7)
	6.174 ± 0.034(6)	8.464 ± 0.039(6)

Table 17

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.

R and S hyoscyamine and derivatives



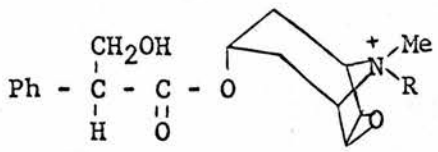
(R)	R	S
H Br$^-$	6.861 ± 0.071(8)	9.380 ± 0.029(5)
Me	7.725 ± 0.052(9)	9.666 ± 0.064(10)
Et	7.061 ± 0.040(6)	8.787 ± 0.040(6)
nPr	6.263 ± 0.058(6)	7.500 ± 0.040(8)
nBu	5.885 ± 0.018(6)	7.087 ± 0.013(6)

Table 18

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.

R and S hyoscine and derivatives



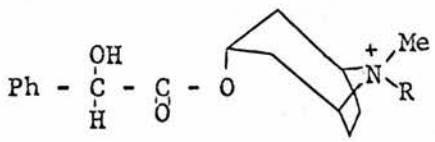
(R)	R	S
H Br⁻	7.572 ± 0.015(12)	9.363 ± 0.062(6)
Me	8.622 ± 0.038(6)	9.702 ± 0.068(9)
Et	7.153 ± 0.036(7)	8.603 ± 0.055(5)
nPr	6.770 ± 0.045(6)	8.269 ± 0.048(6)
nBu	-	7.162 ± 0.059(7)

Table 19

Affinity for receptors in guinea-pig ileum

Values of log K are shown with the standard error and number of results.

R and S homatropine and derivatives

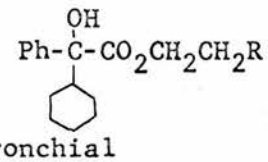


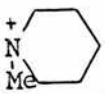
(R)	S	R
H (sulphate)	6.991 ± 0.025(7)	7.441 ± 0.031(7)
Me	7.279 ± 0.017(5)	7.860 ± 0.023(6)
Et	6.774 ± 0.028(6)	6.968 ± 0.027(6)

Table 20

Affinity for receptors in guinea-pig bronchial strip and iris.

Values of log K are shown with the standard error and number of results.



(R)		Bronchial Strip	(+ Hex.)	Iris
$^{+}NMe_3$	S	7.284 ± 0.023(4)		
	R	9.935 ± 0.046(5)	9.818 ± 0.035(4)	
$^{+}NEt_3$	(±)	9.675 ± 0.031(4)		
	(±)calc.	9.704		
	S	7.992 ± 0.023(4)	7.845 ± 0.042(6)	8.006 ± 0.051(7)
	R	10.001 ± 0.055(7)	9.891 ± 0.024(6)	10.148 ± 0.044(6)
	S	7.413 ± 0.051(6)		
	R	9.973 ± 0.024(5)	9.449 ± 0.028(4)	
Hyoscine ethiodide	R	7.268 ± 0.072(4)		
	S	8.793 ± 0.069(4)	8.708 ± 0.059(5)	8.889 ± 0.068(4)

(+ Hex) indicates measurements made in the presence of hexamethonium (2.76×10^{-4}M).

(±) calc. is the value of log K for a racemic mixture calculated from values of log K for the isomers.

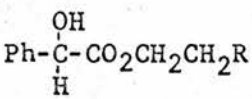
Table 21

Effect of composition on affinity constants (guinea-pig ileum) of enantiomeric mixtures of cyclohexylphenylglycolloylcholine iodide. The percentage of the stronger isomer present in the mixture (y_s%) is shown with the mean estimate of log affinity constant (log K*) ± the standard error and number of results.

y_s%	log K*
0	7.130 ± 0.037 (6)
0.1	7.252 ± 0.023 (4)
0.5	7.570 ± 0.036 (5)
1.0	7.737 ± 0.055 (7)
5.0	8.360 ± 0.033 (6)
10.0	8.642 ± 0.054 (7)
20.0	8.886 ± 0.029 (11)
50.0	9.369 ± 0.026 (5)
100	9.647 ± 0.073 (11)

Table 22

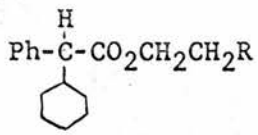
Values of log K (guinea-pig ileum) from table 13 used to calculate log stereospecific index (log S.S.I.) with 95% confidence limits and log K for a racemic mixture ((±) calc). The experimental value of log K for the racemate is also shown with the standard error and number of results.



Onium group (R)	R	S	(±)	(±)calc	log S.S.I.	S.S.I.
$^{+}NMe_3$			5.288 ±0.069(7)			
$^{+}NMe_2Et$			5.893 ± 0.027(7)			
$^{+}NMeEt_2$	6.153	6.044	5.979 ±0.022(7)	6.102	0.229 0.109 0.011	1.3
$^{+}NEt_3$	6.103	6.117	6.086 ±0.022(9)	6.110	0.209 $\bar{1}$.986 $\bar{1}$.763	0.97
	5.974	5.597	5.902 ±0.024(8)	5.825	0.464 0.377 0.290	2.4
	6.368	5.911	6.233 ±0.015(11)	6.197	0.550 0.457 0.364	2.9
	5.674	5.321	5.627 ±0.012(6)	5.533	0.445 0.353 0.261	2.3
	6.021	5.501	5.818 ±0.050(8)	5.835	0.656 0.520 0.384	3.3
Mandelyl tropine	7.441	6.991	7.215 ±0.023(8)	7.272	0.537 0.450 0.363	2.8
Methiodide	7.860	7.279	7.833 ±0.043(5)	7.660	0.649 0.581 0.513	3.8
Ethiodide	6.968	6.774	6.849 ±0.034(10)	6.882	0.281 0.194 0.107	1.6

Table 23

Values of log K (guinea-pig ileum) from table 14 used to calculate log stereospecific index (log S.S.I.) with 95% confidence limits and log K for a racemic mixture ((±) calc). The experimental value of log K for the racemate is also shown with the standard error and number of results.



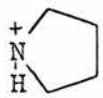
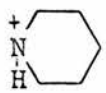
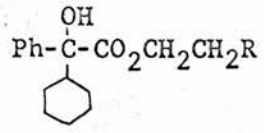
Onium group (R)	(-)	(+)	(±)	(±)calc	log S.S.I.	S.S.I.
$^{+}NMe_2H$	7.952	7.392		7.756	0.717 0.560 0.403	3.6
$^{+}NEt_2H$	8.216	8.000		8.121	0.321 0.216 0.111	1.6
	8.262	7.881		8.112	0.466 0.381 0.296	2.4
	8.115	8.427		8.299	$\bar{0}.749$ $\bar{1}.688$ $\bar{1}.627$	0.49
$^{+}NMe_3$	8.574	8.100	8.438 ±0.046(9)	8.399	0.539 0.474 0.409	3.0
$^{+}NMe_2Et$	8.992	8.500	8.970 ±0.014(6)	8.812	0.599 0.492 0.385	3.1
$^{+}NMeEt_2$	8.849	8.490	8.699 ±0.014(10)	8.705	0.493 0.359 0.225	2.3
$^{+}NEt_3$	8.533	8.431	8.566 ±0.019(8)	8.485	0.156 0.102 0.046	1.3
	8.675	8.301	8.526 ±0.054(8)	8.527	0.451 0.374 0.297	2.4
	8.935	8.568	8.677 ±0.036(9)	8.789	0.440 0.367 0.294	2.4
	8.303	8.301	8.290 ±0.031(7)	8.302	0.046 0.002 $\bar{1}.958$	1.0
	8.151	8.224	8.099 ±0.028(8)	8.188	0.066 $\bar{1}.927$ $\bar{1}.788$	0.8

Table 24

Values of log K (guinea-pig ileum) from table 15 used to calculate log stereospecific index (log S.S.I.) with 95% confidence limits and log K for a racemic mixture ((±) calc). The experimental value of log K for the racemate is also shown with the standard error and number of results.



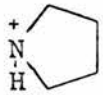
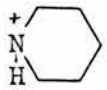
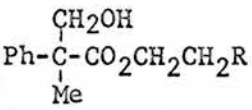
Onium group (R)	R	S	(±)	(±)calc	log S.S.I.	S.S.I.
$^{+}NMe_2H$	8.866	6.977			2.084 1.889 1.694	77.6
$^{+}NEt_2H$	9.299	7.756			1.717 1.543 1.369	34.9
	8.860	7.191			1.833 1.669 1.505	46.7
	9.008	6.935			2.262 2.073 1.883	118.3
$^{+}NMe_3$	9.647	7.~~316~~ 257	9.365 ±0.033(7)	9.348	2.546 2.331 2.116	214.3
$^{+}NMe_2Et$	10.040	7.882	9.804 ±0.042(11)	9.742	2.257 2.158 2.059	143.9
$^{+}NMeEt_2$	10.000	8.150	9.777 ±0.030(7)	9.705	1.974 1.850 1.726	70.8
$^{+}NEt_3$	9.600	7.989	9.482 ±0.030(8)	9.310	1.785 1.611 1.437	40.8
	9.635	7.488	9.473 ±0.026(9)	9.337	2.294 2.147 2.000	140.3
	9.723	7.765	9.588 ±0.047(7)	9.427	2.130 1.958 1.786	90.8
	9.474	7.334	9.215 ±0.044(9)	9.176	2.257 2.140 2.023	138.0
	9.253	7.413	9.081 ±0.047(7)	8.958	1.936 1.840 1.744	69.2

Table 25

Values of log K (guinea-pig ileum) from table 16 used to calculate log stereospecific index (log S.S.I.) with 95% confidence limits and log K for a racemic mixture ((±) calc).



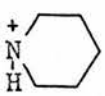
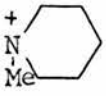
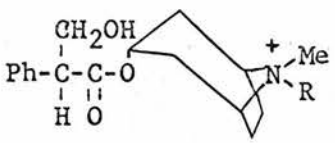
Onium group (R)	(-)	(+)	(±) calc.	log S.S.I.	S.S.I.
	7.354	5.570	7.060	1.874 1.784 1.721	60.8
	7.996	6.126	7.701	2.006 1.870 1.734	74.1
	7.645	5.126	7.345	2.603 2.519 2.395	330.4
	7.452	5.312	7.154	2.236 2.140 2.043	138.0
	8.119	6.058	7.822	2.105 2.061 1.927	115.1
	8.841	6.648	8.543	2.056 2.193 2.330	156.0
	8.478	6.681	8.184	1.902 1.797 1.692	62.7
	8.286	6.607	7.994	1.752 1.679 1.605	47.8
	8.229	6.564	7.938	1.734 1.665 1.596	46.2
	8.289	6.739	8.000	1.642 1.550 1.458	35.5
	8.099	6.406	7.807	1.800 1.693 1.586	49.3
	8.464	6.174	8.165	2.405 2.290 2.174	195.4

Table 26

Values of log K (guinea-pig ileum) from table 17 used to calculate log stereospecific index (log S.S.I.) with 95% confidence limits and log K for a racemic mixture ((±) calc). The experimental value of log K for the racemate is also shown with the standard error and number of results.

R and S hyoscyamine and derivatives



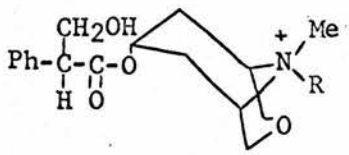
(R)	R	S	(±)	(±) calc	log S.S.I.	S.S.I.
H	6.861	9.380	9.007 ±0.017(12)	9.080	2.726 2.519 2.312	330.4
Me	7.725	9.666	9.454 0.013(4) 9.53* ±0.04(4)	9.370	2.117 1.941 1.765	87.3
Et	7.061	8.787	8.82* ±0.04(4)	8.494	1.853 1.726 1.599	53.2
nPr	6.263	7.500	7.88* ±0.04(4)	7.224	1.385 1.237 1.089	17.3
nBu	5.885	7.087	7.45* ±0.04(4)	6.813	1.251 1.202 1.152	15.9

* Values obtained by Green and others (1972)

Table 27

Values of log K (guinea-pig ileum) from table 18 used to calculate log stereospecific index (log S.S.I.) with 95% confidence limits and log K for a racemic mixture ((±) calc).

R and S hyoscine and derivatives



(R)	R	S	(±)	(±) calc.	log S.S.I.	S.S.I.
H	7.572	9.363	-	9.069	1.890 1.791 1.692	61.8
Me	8.622	9.702	-	9.436	1.274 1.080 0.886	12.0
Et	7.153	8.603	-	8.317	1.590 1.450 1.310	28.2
nPr	6.770	8.269	-	7.982	1.646 1.499 1.352	31.6
nBu	-	7.162	-	-	-	-

DISCUSSION

Discussion

Optical purity

Any investigation of the stereospecificity of the binding of enantiomeric antagonists to receptors depends firstly upon adequate resolution of the antagonist into its optical isomers. This is usually done by recrystallising diastereoisomeric salts to constant melting point and rotation, and then liberating the resolved substance. There is of course no guarantee that resolution carried out in this way is complete. It is therefore not advisable to compare the stereospecificity of related compounds which have been resolved independently. There are however nuclear magnetic resonance techniques being developed which can be used to determine absolutely the enantiomeric composition of optically active substances. There are three NMR methods currently in use:

1) Formation of diastereoisomeric derivatives which have different chemical shifts for certain groups in the enantiomers being studied (Casy, 1971 and references cited therein).

2) The use of an optically active solvent. Here diastereoisomeric collision complexes between the solvent and the solute give rise to chemical shift differences for certain groups in the enantiomers (Casy, 1971 and references cited therein).

3) Addition of optically active rare earth shift reagents to the enantiomeric solution. Chemical shift differences are generated for the various antipodal groups by the formation of loosely bound diastereoisomeric complexes (Whitesides and Lewis, 1970, 1971; Goering and others, 1971).

The third method is the most attractive but attempts to use a Eu^{III}:camphor complex in the present work to determine the optical

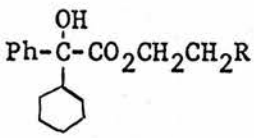
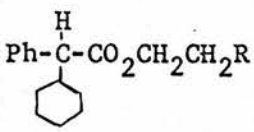
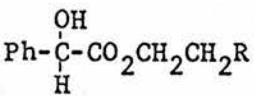
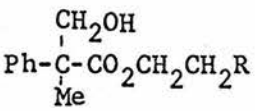
purity of hyoscyamine quaternary compounds have so far failed. Solubility was a major problem because the shift reagent is only soluble in non-polar solvents whereas the quaternaries are not. At the moment, however, these methods seem unable to detect the presence of less than one to two per cent of one isomer in a mixture, and for highly stereospecific compounds this level of accuracy is not adequate for the prediction of true stereospecific indices (Figure 2).

Because of these difficulties the present work has involved synthesis of series of compounds from single sources of resolved material. This may of course not have been completely resolved but all the compounds in the series should be equally incompletely resolved, provided their method of synthesis did not involve racemisation. In the series made the rotations of pairs of enantiomers were, within the limits of measurement, equal and opposite, so the optical isomers should have been equally (probably incompletely) resolved.

Because the experiments with mixtures of cyclohexylphenylglycolloylcholine iodide indicated that these isomers competed with each other and with the agonist it seems likely that the other enantiomeric compounds behave similarly, especially in view of similar results obtained with the enantiomeric forms of benzhexol (Barlow, Franks and Pearson, 1972a). The relationship between optical purity and the observed stereospecific index (page 30) could thus be used to estimate the lowest possible degree of resolution achieved in each series. The highest observed stereospecific index in each series has been used in the calculation and the results are shown in Table 28. It appears that, provided the compounds are appreciably stereospecific, the biological method of assessing the degree of resolution is more sensitive than any of the NMR techniques.

Table 28

Minimum degree of resolution achieved, calculated from the highest stereospecific index observed in a series

Series	Highest S.S.I.	Degree of Resolution %
	220	> 99.55
	3.6	> 78.26
	3.8	> 79.17
	330	> 99.70
Hyoscyamine and derivatives	330	> 99.70
Hyoscine and derivatives	62	> 98.41
Homatropine and derivatives	3.8	> 79.17

A good check on the optical purity of the cyclohexylphenylglycolloyl series is provided by the values of log K obtained by Brimblecombe and others (1971) for the trimethyl ammonium isomers. They found a stereospecific index of 200 (cf 220 in this work) with compounds prepared by a stereospecific route. For the dimethylamino compounds, tested as the hydrochlorides, they obtained a stereospecific index of 100 (cf 78 in this work).

Although the values of the stereospecific index for hyoscyamine and hyoscine indicate a very high degree of resolution those obtained for the quaternary derivatives must be viewed with suspicion. It is well known that hyoscyamine and hyoscine are readily racemised (Will, 1888; Gadamer, 1901; Barrowcliff and Tutin, 1909; Duilius, 1930) and the conditions for quaternisation may cause partial racemisation. For the introduction of groups larger than ethyl, refluxing for several hours was required. A plot of rotation against time of reflux was made for a sample of S hyoscyamine in acetonitrile (the solvent used for the quaternisations) and it was found that the rotation decreased with time. On the other hand, when S hyoscyamine methiodide was refluxed in acetonitrile there was no change in rotation after 42 hrs. If the quaternary salt is formed rapidly, therefore, little racemisation should have occurred so the differences between the stereospecific indices of hyoscyamine hydrobromide and methiodide are likely to be real. With the n-propyl and n-butyl compounds, however, quaternisation is slow and appreciable racemisation will probably have occurred. With the ethyl compounds the observed differences in stereospecific index should be somewhere near the true value.

Although there is some uncertainty about the extent to which any compounds containing the unit $R\text{-}\overset{H}{\underset{R'}{C}}\text{-}CO_2$ may racemise like the

tropic acid derivatives, there is no reason why there should be any racemisation in the derivatives of α-methyl tropic acid and cyclohexylphenylglycollic acid which lack the enolisable hydrogen atom. With these series, at least, the degree of resolution should be constant throughout.

Errors in the estimates of the stereospecific index

Statistically the error in log S.S.I. (log stereospecific index) should depend upon the standard errors of the mean estimates of log K for the isomers and the total number of degrees of freedom ($n_s + n_w - 2$), where n_s is the number of estimates of log K for the stronger isomer and n_w the number for the weaker. The fiducial limits for a chosen level of probability can then be obtained for values of log S.S.I. It is difficult, however, to compare two values of log S.S.I. for significance as each of these is derived from two estimates of log K. Furthermore it has previously been shown that the standard error of the mean value of log K is often an underestimate of the real error.

Comparison of results for the same compounds determined by different people at different times has shown that values of log K which differ from each other by less than 0.1 log units cannot be taken as being really different even when the statistical analysis of the results suggests that they are.(Abramson and others, 1969). This means that values of log S.S.I. which differ by less than 0.2 log units cannot be taken as being really different. This agrees with the comparison of the values of log K for racemic compounds with calculated values for a racemic mixture. Although some values differed by more than 0.1 log units, in no case did they differ by more than 0.2 log units. Unfortunately many of the calculated values of log K for the racemic forms of the hyoscyamine derivatives differ

appreciably (up to 0.8 log units) from the experimental values obtained by Green and others (1972). This is worrying but may be due to the presence of epimeric forms in the quaternary salts (see appendix).

It would seem reasonable then to assume that values of log S.S.I. which differ by less than 0.2 log units are not really different from one another and that differences in log S.S.I. only slightly greater than this should not be taken as indicating definite changes in the stereospecificity of the compounds.

Stereospecific index and receptor structure

The idea of using the stereospecific index to indicate the extent to which receptors in different tissues may be similar is an old one but its value has been limited by the need to use material of the same optical purity in all the biological tests and for activity in the tests to depend only on the interaction between the isomers and the receptors. Recently, Buckner and Patil (1971) have tried to use the technique to compare the structures of the β adrenergic receptors in guinea-pig atria and trachea. Because values of the log S.S.I. for some enantiomers were not significantly different on the two preparations they concluded that the receptors must be similar. In fact, however, their values of log K for the enantiomers have differed up to ten-fold on the two preparations, although the ratio of the activity of the enantiomers has remained the same. The binding of a compound at the two types of receptor is therefore different and if the tests really do measure affinity, the receptors cannot be identical.

In this kind of work, although there is an attraction in using pairs of enantiomers because of their identical physical properties

it should not matter what antagonist is used if measurements are made under conditions of equilibrium. As part of the present work an attempt was made (Barlow, Franks and Pearson 1972b) to see to what extent acetylcholine receptors in the guinea-pig ileum, bronchial muscle and iris resembled each other by measuring log K for 28 compounds, including some enantiomeric pairs. The errors involved in comparing values of log K obtained with different preparations are greater than with estimates of different compounds on the same preparation because of differences in the conditions of the experiments including the time course of the agonist and the method of recording (and the presence or absence of hexamethonium). The results indicated that any differences between the receptors were small and could barely be detected. The stereospecific indices however, (Table 29) seem to follow a trend; the stereospecific index for an enantiomeric pair is always greater in tests on the iris and bronchial strip preparations than in tests on the ileum. This suggests that the receptors are in fact slightly different in structure.

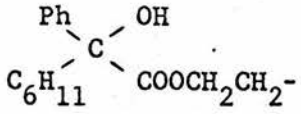
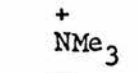
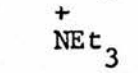
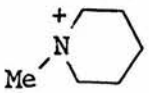
Effect of the structure of the onium group on the stereospecific index

Abramson and others (1969) studied many series of compounds having a range of onium groups, which were antagonists of acetylcholine at postganglionic parasympathetic receptors. They tried to measure the effect of various chemical groups on affinity, and the results suggested that a group had in fact two effects. One, which affected the binding to the receptors of that part of the molecule containing the group, and another which disturbed (and reduced) the binding of distant parts of the molecule (the disturbance term). There is then, within related compounds which act at the same receptors, a constant optimum effect on binding, the (a) term, which occurs when a group is introduced in a molecule which fits ideally, but also variable

Table 29

Stereospecific index

(values are the antilog. of the differences between the values of log K for the enantiomers)

	Ileum		Bronchial strip		Iris
	no hex	hex	no hex	hex	
					
	222	246	448		
	73	41	102	111	139
	149	138	363		
Hyoscine ethiodide		28	33		

hex indicates measurements made in the presence of hexamethonium (2.76×10^{-4}M).

opposing effects on binding, the (d) term, which depend upon the nature of the molecule as a whole.

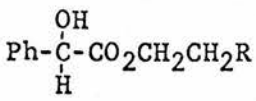
Part of the aim of the present work was to try to find out if changes in the structure of the onium group of compounds containing an asymmetric centre at the opposite end of the molecule, caused changes in the stereospecificity. (This had apparently occurred to a large extent with the resolved forms of benzhexol and procyclidine and their metho and etho salts studied by Duffin and Green (1955)). It was felt that if changes in stereospecificity occurred, then the size of the changes in log S.S.I. between compounds having different onium groups, might give some idea of the change in binding of the main part of the molecule to the receptors as the onium group was altered, i.e. the change in the disturbance term.

The values of log S.S.I. do in fact vary within the series tested, and the size of the larger variations is well outside the limits of experimental error (Table 30). It is interesting that although the α-methyltropic, cyclohexylphenylglycollic, hyoscine and hyoscyamine series are highly stereospecific (log S.S.I. up to 2.5, S.S.I. up to 330) and the mandelic and cyclohexylphenylacetic series are apprently not very stereospecific (log S.S.I. up to 0.6, S.S.I. up to 4), the overall change in log S.S.I. observed in any one series (except the incomplete series of mandelic esters) is approximately 0.8 log units. This suggests a change in binding of the order of 4 kJ $mole^{-1}$.

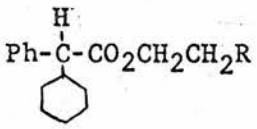
If log S.S.I. is plotted against onium group size (Figure 3) certain trends are apparent. In series which have two large groups attached to the asymmetric carbon atom, an increase in onium group size, within one onium group type, nearly always leads to a drop in log S.S.I. In the series with only one large group attached to the

Table 30

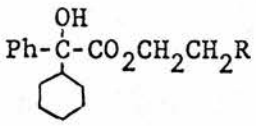
Range of the values of log S.S.I. within the series



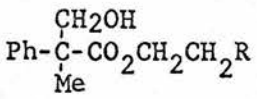
(A)



(B)



(C)



(D)

Series

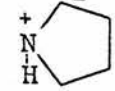
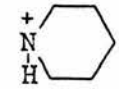
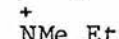
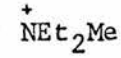
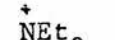
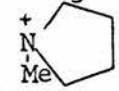
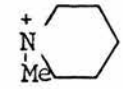
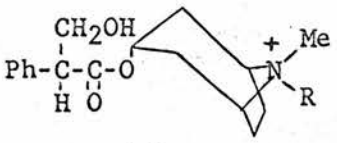
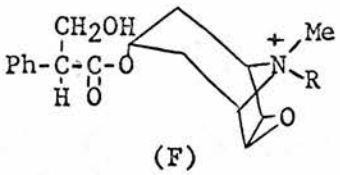
Onium group (R)	(A)	(B)	(C)	(D)
	-	0.6	1.9	1.8
	-	0.2	1.5	1.9
	-	0.4	1.7	2.5
	-	-0.3	2.1	2.1
	-	0.5	2.3	2.1
	-	0.5	2.2	2.2
	0.1	0.4	1.9	1.8
	0	0.1	1.6	1.7
	0.4	0.4	2.1	1.7
	0.5	0.4	2.0	1.6
	0.4	0	2.1	1.7
	0.5	-0.1	1.8	2.3
Overall range	0.5	0.9	0.8	0.9

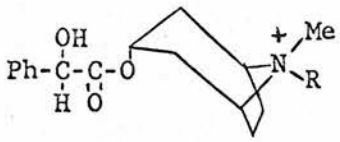
Table 30 (cont'd)



(E)



(F)



(G)

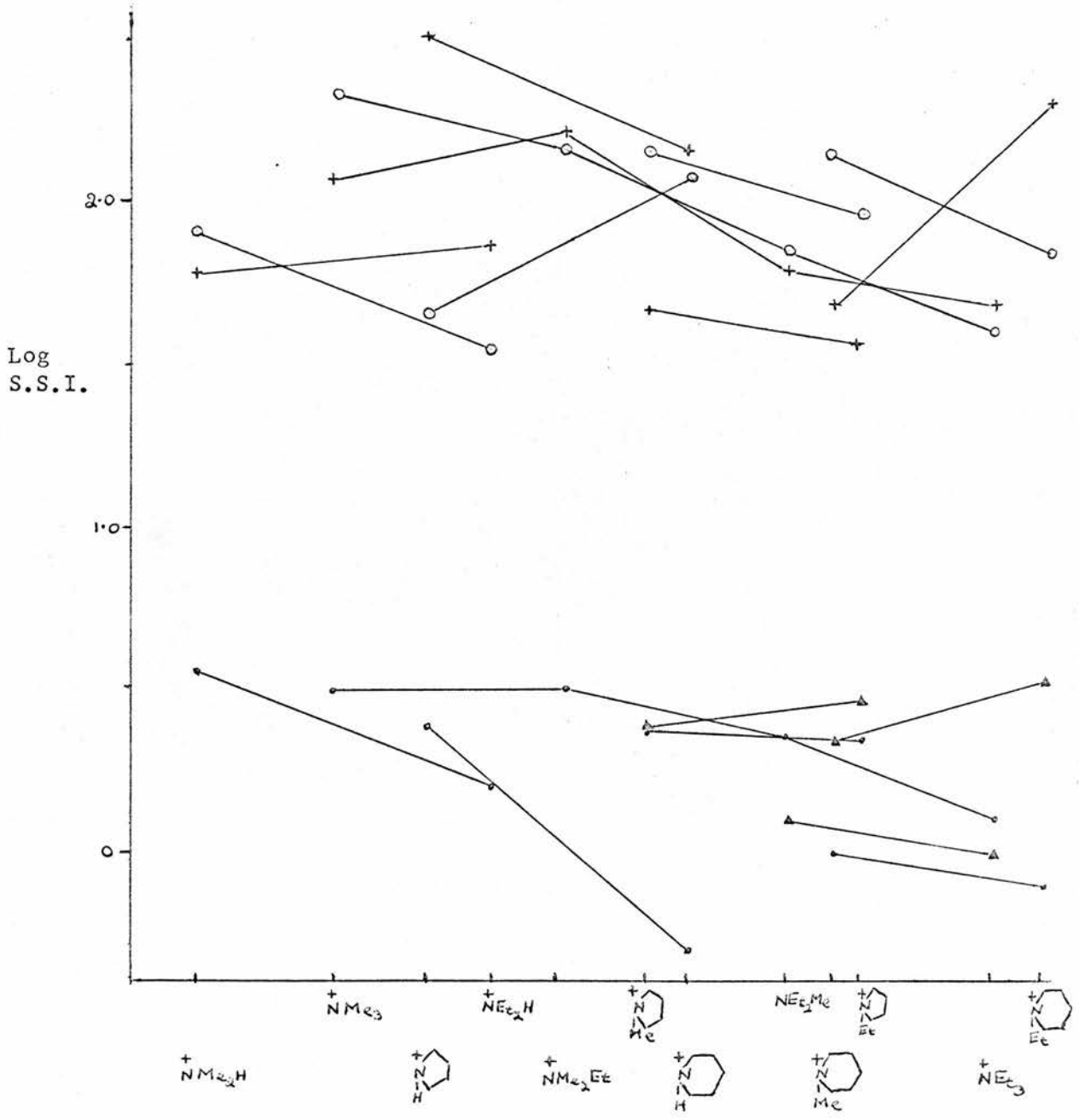
	Series		
	(E)	(F)	(G)
(R)			
H	2.5	1.8	0.5
Me	1.9	1.1	0.6
Et	1.7	1.5	0.2
Overall range	0.8	0.7	0.4

Figure 3

Values of log S.S.I. plotted against quaternary group size (based on results of Lowe and Rendall, 1970). Tertiary groups arranged arbitrarily.

• cyclohexylacetic, ○ cyclohexylglycollic,

▲ mandelic, + α-methyltropic esters.



asymmetric carbon atom, an increase in onium group size, within one onium group type, nearly always leads to an increase in log S.S.I.

Suppose that the free energies of adsorption of a particular pair of enantiomers in a series are A_w for the weaker isomer and A_s for the stronger. If the onium group is increased in size there should be increased facilities for the binding of each enantiomer of (a), but these will be offset by the disutrbance terms (d_w) and (d_s), so the free energies of adsorption will be $A_w + (a) - (d_w)$ and $A_s + (a) - (d_s)$ respectively. The values of log S.S.I. will therefore be changed from

$$\frac{A_s - A_w}{2.3\ RT} \quad \text{to} \quad \frac{A_s + (a) - (d_s) - A_w - (a) + (d_w)}{2.3\ RT}$$

so that $$\Delta \log S.S.I. = \frac{-(d_s) + (d_w)}{2.3\ RT}$$

For instance when the onium group in the esters of phenylcyclohexl-glycollic acid is changed from trimethyl to triethyl, the value of log S.S.I. decreases from 2.3 to 1.6 which means that the change in the disturbance term going from trimethyl to triethyl in the more active isomers is greater than the corresponding change in the less active isomers.

With the series containing only one large group on the asymmetric carbon atom, however, increase in size usually does not lead to decrease in stereospecificity. For instance the change from methyl piperidyl to ethylpiperidyl reduces log S.S.I. from 2.1 to 1.8 in the cyclohexylphenylglycollic series but increases it from 1.7 to 2.3 in the α-methyltropic series. This means that in the

latter series the change in the disturbance term is greater in the weaker isomers. In these circumstances (d_w) cannot be ignored any more than (d_s) and consequently values of log S.S.I. can at best only yield information about differences in disturbance terms.

Biggest effects of groups on affinity

If values of log K are inspected, it is possible to observe the biggest effect that a particular change in structure has upon affinity and this may approximate to the effect (a) of a group when no rearrangement occurs. In effect there is no special merit in using stereospecific indices and more information may be obtained by using the actual values of log K (from which log S.S.I. is derived), particularly because these can be compared with other estimates of log K to observe the net effects of groups on affinity.

The overall range, expressed as Δ log K, of the effects produced by altering the onium group in all the compounds tested here and by Abramson and others (1969) is shown in Table 31. The maximum effects of the various groups, relative to the trimethyl analogue are the best estimates that can be obtained for the probable size of the affinity changes (a) produced by the groups in these particular series, i.e. these values are obtained when the disturbance terms are at their smallest.

A similar treatment of the values of log K can be used to indicate the range of effects of the groups at the other end of the molecule (Table 32). Again the maximum values obtained should approximate to the affinity which a group can contribute when it is in an 'ideal fit' position. The effects of these groups (on binding) are much greater than the effects of onium group composition.

Table 31

Range of the effect of the composition of the onium group on log K, based on all the values obtained here and by Abramson and others (1969)

	$^{+}NMe_2Et$	$^{+}NEt_2Me$	$^{+}NEt_3$				
Maximum increase (a)	0.722	0.893	1.252	0.551	1.035	1.082	0.992
Minimum increase	0.036	0.076	-0.303	-0.227	-0.157	-0.477	-0.499
Disturbance term (d)	0.686	0.817	1.555	0.778	1.192	1.559	1.491

	$^{+}NMe_2H$	$^{+}NEt_2H$		
Maximum increase (a)	-0.280	0.499	-0.066	0.327
Minimum increase	-0.781	-0.358	-0.932	-0.746
Disturbance term (d)	0.501	0.857	0.866	1.073

The difference between log K for the compounds and that for the trimethyl analogues is shown.

Table 32

Range of the effects of various groups on log K, based on all the values obtained here and by Abramson and others, (1969)

			△ log K		
			Maximim increase (a)	Minimum increase	Disturbance term (d)
Effect of replacing					
H	by	Ph	3.5	0.6	2.9
H	by		3.9	0.9	3.0
H	by	OH	1.4	-1.5	2.9
$-CH_2-CH_2-$	by	$-\overset{\overset{O}{\|}}{C}-O-$	0.7	-0.6	1.3
$-\overset{OH}{\overset{\|}{C}}-\overset{H}{\overset{\|}{}}$	by	$-\overset{CH_2OH}{\overset{\|}{C}}-\overset{Me}{\overset{\|}{}}$	2.4	0.5	2.9
$-\overset{OH}{\overset{\|}{C}}-\overset{H}{\overset{\|}{}}$	by	$-\overset{CH_2OH}{\overset{\|}{C}}-\overset{H}{\overset{\|}{}}$	1.9	-0.1	2.0

The maximum possible effects which the groups have on log K can be used to predict the optimum log K of compounds containing these groups, and by comparing the calculated value of log K with the experimental estimate the total disturbance terms can be found. If n-pentyl trimethyl ammonium is used as the parent compound then values of log K, for compounds derived by substitution with the various groups shown in Table 32, can be worked out. n-Pentyltrimethyl-ammonium should be a suitable reference compound because it is likely that the flexibility of the methylene chain ensures maximum fit to the receptor, i.e. the disturbance term should be small.

Example. The optimum log K for phenylacetylcholine $PhCH_2CO_2CH_2CH_2\overset{+}{N}Me_3$ should be given by

1)	log K for n-pentyltrimethylammonium	3.7
	+	
2)	Δ log K for H replaced by Ph	3.5
	+	
3)	Δ log K for $-CH_2CH_2-$ replaced by $-CO_2-$	0.7
	=	7.9

The experimental value is 4.5 so the total disturbance term must be 3.4 log units.

Estimates of the size of disturbance terms

Similar calculations were made for all the trimethyl ammonium compounds which have been tested here and by Abramson and others (1969). The values of the overall disturbance terms seem to fall into three distinct ranges, which are apparently dependent upon the structure of the compounds. This is not surprising because the size of the disturbance terms should depend upon the ease with which the molecule can rearrange its binding to the receptors. This in turn

Table 33

Values of the overall disturbance terms (produced by various substitutions) expressed as differences in log K

	Overall disturbance term Δ log K
1) Introduction of -C(=O)-O- in place of -CH_2-CH_2- along with a) Ph or  in place of H or b) Ph and  in place of 2H	3.5
Also introduction of 2Ph or 2  in place of 2H in n-pentyl chain	
2) Introduction of -C(=O)-O- in place of -CH_2-CH_2- along with 2Ph or 2  in place of 2H	4.5
3) Introduction of Ph or  in place of H in n-pentyl chain	2.1

is related to the flexibility of the molecule, and to the nature of its substituent groups, and those in the receptor with which they interact. (Table 33)

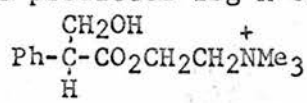
It can be seen that the introduction of large groups at the end of a flexible pentyl chain causes much less disturbance of binding than does similar substitution in the more rigid ester containing analogues. The introduction of two phenyl or two cyclohexyl groups causes more disturbance than the introduction of one phenyl along with one cyclohexyl group. Abramson and others (1969) explained this by postulating that the receptor has two sites which bind preferentially to phenyl and cyclohexyl respectively, and so two phenyl or two cyclohexyl groups cannot be accommodated as readily as one phenyl plus one cyclohexyl group. Apparently the disturbance in binding which occurs when one phenyl group is introduced allows further substitution by a cyclohexyl group to take place without causing additional disturbance. From the values of the disturbance terms which have been found it appears that substitution by two phenyl or two cyclohexyl groups in an n-pentyl chain causes approximately the same disturbance as the introduction of one phenyl, one cyclohexyl, or one phenyl plus one cyclohexyl, in the corresponding ester linked compound.

Predicted values of log K

Although the values for the optimum affinity changes and the disturbance terms which have been found are limited to very closely related compounds, tested at one type of receptor, they at least provide a guide for the calculation of the affinity of new compounds of similar structure.

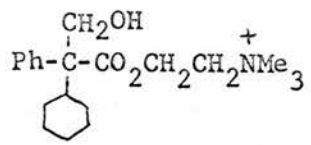
Examples.

The predicted log K of the more active isomer of tropyl-

choline 

is 3.7 + 3.5 + 0.7 + 1.4 + 1.9 - 3.6 = 7.6

and that of the more active isomer of α-cyclohexyl-α-phenyl-β-hydroxypropionylcholine



is 3.7 + 3.5 + 3.9 + 0.7 + 1.4 + 1.9 - 3.6 = 11.6

It would be interesting to see if experimental estimates of log K agree with these predicted values, particularly for the latter compound because this value of log K is exceptionally high. Note that in compounds where the introduction of groups leads to the formation of an asymmetric centre the predicted affinity is that of the enantiomer which binds more strongly to the receptors, because this approximates more closely to the 'ideal fit' case. The value of log K for the racemic compound cannot, of course, be more than 0.3 log units less than this value, provided the enantiomers compete with one another and with the agonist.

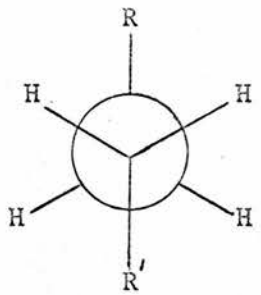
Conformation of the antagonists

Part of the disturbance term observed in a particular compound type may be due to conformational changes which take place in the molecule as groups are added. All the compounds tested can be considered to be substituted ethanes

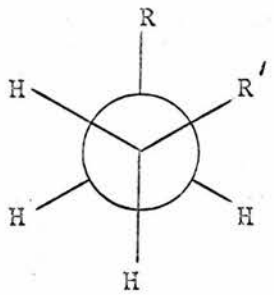
$$R - CH_2 - CH_2 - R'$$

where R = $CH_3(CH_2)_2$, $PhCH_2CO_2$-, etc. and R′ = NMe_3, NMe_2Et etc.

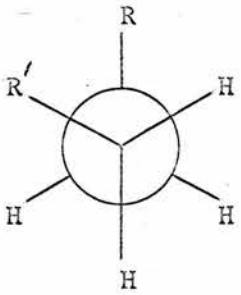
Abraham and Gatti (1969) have used the vicinal H-H coupling constants about the bimethylene bond observed in the NMR spectra of substituted ethanes to estimate the energy difference between gauche and trans forms.



trans

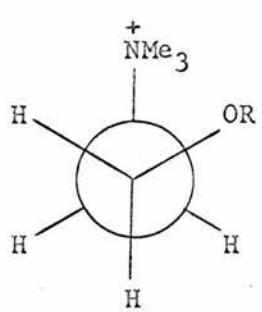


gauche

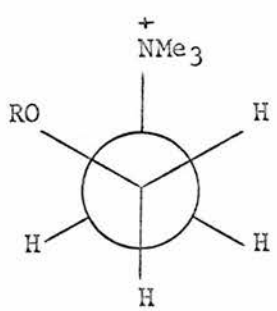


gauche

When, for example, R and R′ = I, the energy difference was 11 kJ mole^{-1}. A similar treatment, in conjunction with ^{14}N-β-C-H vicinal coupling data, has been used by Partington and others (1972) to determine the relative populations of the gauche and trans forms of acetylcholine and many related compounds in deuterium oxide. The bimethylene bond of acetylcholine and related compounds provides a useful reference axis about which conformations of the other 'pharmacophoric groups' may be assigned. They have shown that acetylcholine and most related compounds, including the antagonist benziloylcholine, which have an oxygen atom attached to the β carbon atom ($-\underset{\beta}{CH_2}-\underset{\alpha}{CH_2}-\overset{+}{N}$) exist predominantly in a gauche conformation.



gauche



gauche

The average observed population distribution, gauche:trans, is approximately 95:5 compared with a ratio of 67:33 which would be expected if there were no conformational preference. The free energy required to produce this distribution is at least 7.5 kJ $mole^{-1}$ (This corresponds to a change in log K of approximately 1.3 log units). In contrast phenylethyltrimethylammonium, cyclohexylethyltrimethylammonium and other compounds lacking an oxygen function were shown to be predominantly trans. It may be then that the n-pentyl compounds studied by Abramson and others (1969) are predominantly trans, while the oxygen containing ethers, and esters are predominantly gauche. Part of the difference in disturbance terms between the pentyl and the ester-linked compounds may then be due to the conformational energy difference between the two forms. Without NMR evidence it is uncertain whether the introduction of groups has an effect upon conformation and in this situation the use of enantiomeric pairs of compounds has the advantage that any conformational changes which take place should be the same in both enantiomers. The difference in the disturbance terms should therefore be only a measure of the altered interaction of the groups in the molecule with those in the receptor, caused by reversal of configuration.

It has been shown by NMR spectroscopy (see Appendix) that mandelylcholine and α-methyltropylcholine are in fact approximately 100% in the gauche conformation. A full NMR study has not been possible and so the conformations of the other antagonists are uncertain, but from the above results it seems likely that all these acetylcholine like antagonists exist predominantly in the gauche conformation.

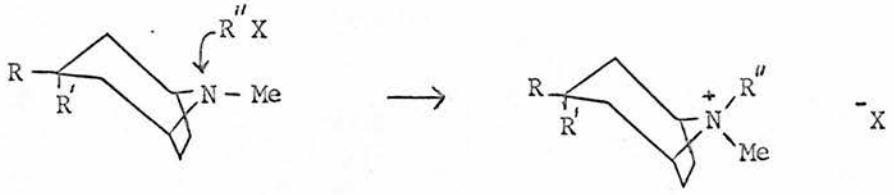
APPENDIX

Appendix

Investigation of the presence of epimeric forms in some quaternary derivatives of atropine by NMR spectroscopy

Quaternisation of tropine-like compounds which have a nitrogen atom in a bridged ring structure may give N-epimeric products. If attack is primarily axial then the major product will have the entering group axial, whereas if attack is predominantly equatorial the major product will have the entering group equatorial.

Axial attack:



Equatorial attack:

It has recently been established conclusively by NMR spectroscopic and X-ray crystallographic studies that the predominant pathway for ethylation, methylation, alkoxycarbonylmethylation and other quaternisations of tropine, pseudotropine, tropinone and several related compounds is by equatorial attack (Fodor and others, 1971; de la Camp and others, 1972). The ratios of entering group equatorial:entering group axial for these compounds lay in the range 70:30 to 90:10.

There was a possibility then that the quaternary hyoscyamine derivatives tested in the present work consisted of epimeric mixtures. It seemed likely that attack would have been mainly equatorial, but it was not known whether recrystallisation had been sufficient to

remove the minor N-axial product.

The 100 MHz NMR spectra of recrystallised samples of atropine metho, etho and n-propyl iodides were therefore run in D_2O. The N-Me signals relative to internal DSS were sharp singlets with the following chemical shifts:

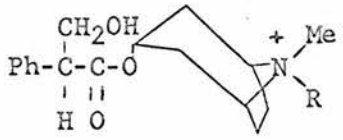
Methiodide	Ethiodide	n-Propyl iodide
312 and 304 Hz (δ 3.12 and 3.04)	302 Hz (δ 3.02)	304 Hz (δ 3.04)

The absence of further N-Me signals in the spectra of the purified etho and n-propyl iodides showed that only one epimeric form was present. When atropine was quaternised with ethyl iodide exactly as before and the total crude quaternary product was collected, its NMR spectrum showed two N-Me signals with peak heights having a ratio of approximately 6:1 at 300 Hz and 294 Hz (δ 3.00 and 2.94) respectively. The axial and equatorial N-Me signals of the epimers of tropine ethobromide studied by de la Camp and others (1972) were coincident at δ 3.02 in D_2O, but were separated at δ 3.01 and 2.98 in DMSO-d_6. These authors confirmed the original conclusion of Closs (1959) that the equatorial N-Me signals of simple tropane derivatives come to resonance at higher field than the corresponding N-Me axial adsorptions.

It is therefore fairly certain that the low field N-Me signals observed in the quaternary derivatives of atropine arise from the axial N-Me group, but this is dependent on the assumption that in atropine the attacking group enters the equatorial position, as it has been shown to do in the other tropine like compounds examined. The chemical shift of the axial N-Me of atropine methiodide is at lower field than the corresponding signals for the ethyl and n-propyl derivatives, because these protons are shielded by the weak electron

donating effects of the ethyl and n-propyl groups.

The quaternary hyoscyamine derivatives which were tested in the present work were all recrystallised to constant melting point, and from the NMR results it is concluded that these derivatives are of one epimeric form; that which is the major product of quaternisation and has the group (R) equatorial:



The biological activity of the epimers having the group (R) axial may be different and if the compounds tested by Green and others (1972) contained some of this form then this might explain the difference between the values of log K found by them for some atropine quaternary derivatives and the calculated values of log K for a racemic mixture based on values of log K for the enantiomeric forms of hyoscyamine found in the present work.

Determination of the preferred conformation of some of the antagonists in D_2O by NMR spectroscopy

An attempt has been made to determine the preferred conformations in D_2O of mandelyl, α-methyltropyl, cyclohexylphenylacetyl and cyclohexylphenylglycolloylcholine by NMR spectroscopy. The trimethyl ammonium derivatives were chosen because it was hoped that $\underline{N}\text{-}\beta\text{-}C\text{-}\underline{H}^{14}$ vicinal coupling constants might be observed as first order splittings and that estimates of the proton-proton vicinal constants might be made to provide two simple means of determining the preferred conformation about the $R\text{-}CH_2\text{-}CH_2\text{-}N$ bond. These methods involve use of the Karplus relationship which relates the magnitude of the spin coupling

between nuclei on adjacent carbon atoms to the dihedral angle between them. The 100 MHz spectra in D_2O at 30°C were recorded but only the mandelic and α-methyltropic derivatives were sufficiently soluble to provide spectra which could be used for the analyses. The spectra of the soluble derivatives showed AA'XX' spin multiplets for the bimethylene protons. In each case the lower field half of the second order spin system was broadened by additional ^{14}N coupling and was therefore assigned to the -O-$\underline{CH_2}$- protons. It is well known that three-bond $^{14}\underline{N}CC\underline{H}$ coupling is larger (1-3 Hz) than two bond $^{14}\underline{N}$-C$\underline{H}$ coupling (0-0.5 Hz) (Culvenor and Ham, 1966 and references cited therein).

$^{14}\underline{N}$-β-C-$\underline{H}$ coupling constants. Comparison of the higher and lower field methylene signals (R-$\underline{CH_2}$-$\underline{CH_2}$-N) as described by Partington and others (1972) gave an approximate $^{14}\underline{N}$-β-C-$\underline{H}$ coupling constant, even though the low field signal was not well resolved. This was probably due to relaxation broadening effects which might be reduced or removed in future experiments by running the spectra at $\simeq 90^{\circ}$ (see Inch and others, 1970)

Approximate $J_{1,3}$ and $J_{1,4}$ coupling constants. Examination of the well resolved higher field ($\underline{CH_2}$N) methylene signal allowed estimation of approximate values for N and L, where $N = J_{1,4} + J_{1,3}(1)$ and $L = J_{1,4} - J_{1,3}(2)$ (Garbisch, 1968). The signs of vicinal proton-proton coupling constants are always the same but in order to determine the sign of L i.e. to allow correct labelling of the J's in equation (2) the approach of Culvenor and Ham (1966) was used. This involves use of an empirical relation between N, L and the electronegativity of the groups attached to either end of the methylene chain.

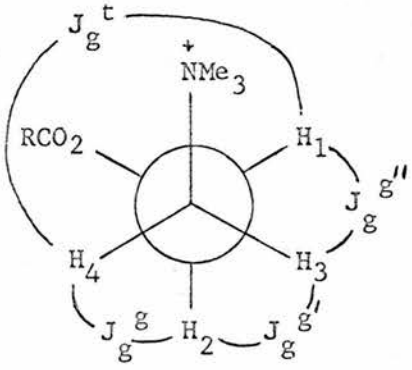
$$\frac{1}{2}N + \frac{1}{6}L = J_{AV} = 17.97 - 0.80 \sum E$$

For compounds like acetylcholine where the atoms attached to the bimethylene chain are oxygen and nitrogen $\sum E = 15.3$ using Huggins (1953) electronegativity values. The sign of L is that which causes $\frac{1}{2}N + \frac{1}{6}L$ to approach the value for J_{AV} more closely, and thus L is positive for both the compounds examined. In these acetylcholine like compounds positive values of L indicate a predominantly gauche conformation (Culvenor and Ham, 1966). Substitution of values for N and L into the simultaneous equations (1) and (2) gives $J_{1,3}$ and $J_{1,4}$ (see later). These values can then be used (Partington and others 1972; Ison and others, 1972) to calculate the population distribution of the preferred conformations by substitution in the equations:

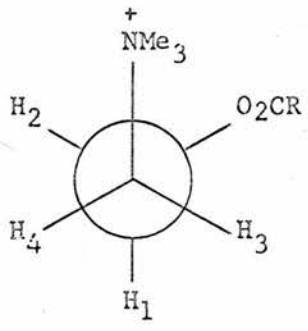
$$J_{1,3} = a(J_g^{g} + J_g^{g''}) + bJ_t^{t} \qquad (3)$$

$$J_{1,4} = a J_g^{t} + aJ_g^{g'} + bJ_t^{g} \qquad (4)$$

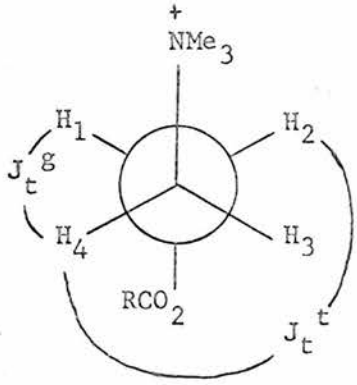
where a represents the fraction of rotamers in each gauche conformation and b the fraction in the trans conformation and $2a + b = 1$. J_g^{g} etc. (see diagrams below) refer to the individual coupling constants within the classical gauche and trans rotamers and are values calculated from the electronegativities of the substituent groups (Abraham and Gatti, 1969).



gauche



gauche



trans

Equation (4) has not been used to calculate rotamer populations because it is less sensitive to changes in a and b (J_t^g is approximately equal to $\frac{1}{2}(J_t^g + J_g^{g'})$) (Partington and others, 1972).

Values of coupling constants (Hz) and preferred conformation

	$J_{14_{NH}}$	$J_{1,4}$	$J_{1,3}$	gauche %	trans %
α-methyltropylcholine	2.5	7.3	1.7	100	0
mandelylcholine	2.5	8.5	2.5	100	0

Both the $J_{14_{NH}}$ coupling constant observed and the vicinal proton-proton coupling constants agree well with the values obtained by Partington and others (1972) for the many acetylcholine like compounds which were shown to be approximately 100% gauche. These results provide some direct evidence that the complete series of antagonists probably exist predominantly in the gauche conformation which may be relevant to the formation of the drug:receptor complex (see page 45).

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